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Intestinal Absorption of *B. prodigiosus*.*

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(Introduced by W. H. Manwaring.)

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Nedzel and Arnold¹ report experimental evidence that massive absorption of viable bacteria may take place from the normal canine intestinal contents into the blood stream, an appreciable "cyclic circulation" of their test bacteria (*B. prodigiosus*) to and from certain mucous cavities. They report that this normal "circulation" is increased as a result of the local action of egg-white.

In attempting to confirm their experimental data, we have been forced to the conclusion that while massive duodenal absorption of their test microorganisms may occasionally take place through the presumably normal intestinal mucosa, in approximately 90% of all normal dogs thus far tested by us, the intestinal absorption was much less rapid than their data would indicate. In our hands only an occasional femoral blood sample showed one or more viable microorganisms per cubic centimeter. (Table I.)

Counts approaching their femoral artery data, however, were ob-

* The prodigiosus culture used in most of these tests was kindly furnished by Dr. Arnold's laboratory.

¹ Nedzel, A. J., and Arnold, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 353.

TABLE I. Femoral Blood Samples.

The abdomen was opened under ether anesthesia, and a small incision made in the stomach wall. Through this incision a soft catheter was passed into the duodenum and 50 cc. warm Ringer's solution containing one 24-hour agar slant of *B. prodigiosus* were injected. Blood samples were withdrawn at stated intervals from a superficial femoral artery. The table records prodigious counts per cc. in these samples (— = no sample). The average or composite count was obtained by disregarding Dog No. 1, which was evidently atypical.

Dog No.	Time interval in minutes after duodenal injection.												
	0	5	10	15	20	25	30	35	40	45	50	55	60
1	0	670	80	40	290	0	0	0	0	0	390	10	—
2	0	3	0	2	0	0	0	0	0	1	0	4	0
3	0	1	2	1	8	0	0	1	2	6	3	2	0
4	0	0	0	—	0	0	0	—	—	—	—	—	—
5	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	1	0	7	1	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	1	0	0
9	0	0	0	0	1	0	1	0	0	—	—	—	—
10	0	7	0	4	2	0	0	0	0	—	—	—	—
Average	0	1	0	1	1	1	0	0	0	1	1	1	0

tained from periodic or continuous blood samples withdrawn from the portal vein. (Dogs 11 to 14, Table II.) These samples were obtained by means of a glass cannula inserted into one of the portal collaterals. No very material increase in this normal portal count was observed, however, by substituting egg-white for Ringer's solution in the injection mass (Dogs 15 to 18, Table II).

We have made a few introductory tests of the effects of certain local pathological conditions on the normal duodenal absorption rate. Much to our surprise, slight mechanical abrasions to the duodenal

TABLE II. *Portal Blood Samples.*
 In Dogs Nos. 11 to 14 inclusive the initial bacterial suspension was made in Ringer's solution; in Dogs
 Nos. 15 to 18 in egg-white.

Dog. No.	Time interval in minutes after duodenal injection.														39
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	
11	0	132	15	525	50	80	2	0	1	0	40	—	—	—	—
12	0	0	0	0	0	130	9	2	0	0	—	—	—	—	—
13	0	0	6	0	9	300	—	—	—	—	—	—	—	—	—
14	0	1	511	150	0	0	0	0	0	56	0	88	—	—	—
15	0	3	16	—	0	1	0	0	80	0	640	—	—	—	—
16	0	2	58	530	6	0	4	20	340	0	0	7	—	—	—
17	0	0	0	8	0	0	0	0	0	30	0	0	—	—	—
18	0	—	—	—	0	0	—	1	380	40	0	7	67	50	—
Average	0	20	87	200	8	64	25	33	114	18	113	25	—	—	—

mucosa did not increase the usual portal count, nor were important changes in absorption rate produced by previous sensitization or immunization with *B. prodigiosus* or its autolysate. Work on the effects of specific immunization, however, is being continued.

A marked increase in normal duodenal absorption was noted, however, as a result of local passive congestion (mechanical obstruction of portal vein). In our short series of tests, the average

portal count rose to 12,000 *B. prodigiosus* per cc. by the end of 15 minutes. The most rapid absorption thus far recorded by us, however, was during acute anaphylactic shock (horse proteins). In one test, for example, the portal count rose within 10 minutes to over 1,000,000 viable microorganisms per cc. with over 100,000,000 colonies (agar plate) per gm. of liver emulsion at the end of the test (30 minutes).

This finding suggests a hitherto unemphasized factor in the clinical picture of anaphylaxis and related shock conditions. It also suggests a conceivable source of error in the production of specific antisera, any shock condition during immunization conceivably vitiating serum specificity.

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Reduction of Nitrates and Nitrites by Representatives of the *Brucella* Group.

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The *Brucella* group is generally believed to lack the ability to reduce nitrates. Bergey¹ characterizes both *Br. abortus* and *Br. melitensis* as being non-reducers. Duncan and Whitby² record similar findings. Evans,³ Topley and Wilson⁴ and Lustig and Vernoni⁵ report that nitrates are occasionally reduced.

An investigation was made to determine the factors which influence nitrate reduction and the cause of reported irregularities. A medium of the following composition was used: Peptone 2 gm., beef extract 1 gm.; NaCl 3 gm.; agar 2 gm.; H₂O 1000 cc. The reaction was adjusted to pH 6.8. The semisolid consistency favors the growth of the *Brucella* and accentuates the zone phenomenon, particularly when inoculated with a dilute suspension of cells. The majority of the *Brucella* grow in limited zones 2 to 6 mm. below

¹ Bergey, "Determinative Bacteriology," 1930, **3**, 366.

² Duncan and Whitby, "A System of Bacteriology," 1930, **5**, 395.

³ Evans, *J. Infect. Dis.*, 1918, **22**, 580.

⁴ Topley and Wilson, "Principles of Bacteriology and Immunity," 1929, **1** 508.

⁵ Lustig and Vernoni, *Handb. d. pathogenen Mikroorganismen*, 1928, **4**, 520.

the surface. The *abortus* type* is usually the deepest, the *suis** are nearest the top and the *melitensis** are intermediate. Upon the addition of 0.2% KNO_3 to the medium, a reversal of positions is observed. Now the *suis* types multiply throughout the medium in the form of a pseudo-anaerobic growth, the *abortus* do so to a less extent but the growth zone of the *melitensis* is confined to a few mm. below the surface. The two former varieties are apparently able to obtain their oxygen requirements from the disintegration of nitrates.

Every strain of a collection of 425 *Brucella* tested was found to reduce nitrates vigorously. The presence of nitrites, the disappearance of nitrates and the formation of gas were observed as criteria of the reduction. Frequently 0.1% KNO_3 is quantitatively reduced in 2 days. There is very little tendency for nitrites to accumulate because they are simultaneously reduced, which may account for the apparently negative results reported in the literature. Many strains destroy 0.2% KNO_3 as well as the intermediate nitrites in 4 days. Every strain tested was able to cause the disappearance of 0.01% KNO_2 in 5 days; some destroyed 10 times as much during the same interval. A higher concentration of nitrite has a differential toxic effect. The *abortus* and the *melitensis* types are inhibited by 0.2% KNO_2 while the *suis* continue to multiply in this concentration. The latter variety is the most active nitrate and nitrite reducer. The *melitensis* types reduce nitrites more rapidly than the *abortus*.

Semisolid media expedite nitrate reduction. The beneficial effect is attributed to the improved growth and the favorable O/R potential which results from the partial exclusion of atmospheric oxygen. Nitrate reduction is more rapid in semisolid media than in broth or on agar slants. The multiplication of *Brucella* in semisolid peptone media normally causes the O/R potential to drop to near rH 11.5 at pH 6.8. The presence of nitrates retards this drop until their reduction is complete. The stabilizing influence on the O/R potential indicates an activation of nitrate oxygen.

Succinic, lactic and citric acids, glucose, xylose, galactose and arabinose accelerate nitrate reduction. Under these conditions nitrogen gas is liberated by some strains. Most of the *suis* and *abortus* produce gas but the *melitensis* types were found to be non-gas-producers under identical conditions. The influence of these carbon compounds on nitrate reduction offers further proof that they are attacked by the *Brucella*. Quantitative tests show that the presence of

* The types have been determined by extensive dye tolerance and H_2S tests, the results of which are now in press.

nitrates materially increases the utilization of glucose by all varieties.

Studies in synthetic media gave no evidence that nitrates can supply the nitrogen requirements. Nor did the addition of increasing amounts of utilizable nitrogen compounds effect nitrate reduction any more than could be accounted for by the multiplication rate. Compounds containing reduceable sulphur retard nitrate reduction. Cystine and thiosulfates in concentrations which are ordinarily tolerated become toxic in conjunction with nitrates.

Iodides exert an antagonistic or synergetic inimical action. Nitrate reduction is facilitated by 0.2% KI. However, 1.0% KI in media with 0.2% KNO_3 inhibits multiplication although 2.0% KI is tolerated in the absence of nitrates. The *abortus* and *melitensis* are more sensitive in this respect than are the *suis* types. The toxic effect is thought to be due to an accumulation of nitrites from the rapid reduction of nitrates. In media containing 0.2% each of KNO_3 and KI the nitrates quickly disappear. The test is of some differential value. Every *suis* type produced an abundance of nitrogen gas to the exclusion of the other 2 varieties when grown in such a medium.

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Cross Immunization Experiments with the Poliomyelitis Virus and that of Encephalomyelitis in Horses.

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During the summer and fall of 1930 and 1931, an epizootic of an acute encephalomyelitis broke out among the horses and mules in the rural districts of California.¹ Meyer, Haring and Howitt² report the recovery of a filterable virus as the causative agent and describe hemorrhage, oedema and acute inflammatory lesions in the brain with slight involvement of the cord. Since there were certain similarities between the equine malady and human poliomyelitis and since outbreaks of both forms chanced to occur simultaneously dur-

¹ Haring, C. M., Howarth, J. A., and Meyer, K. F., Univ. Calif. Agri. Exp. Station, 1931, Circular 322, 1.

² Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, 74, 227.

ing 1930 in California, a series of cross immunization experiments were undertaken with both viruses.

Serum was obtained from each of 11 monkeys that had recovered from experimental poliomyelitis, either entirely or with muscular atrophy of certain extremities. According to previous tests these serums neutralized the poliomyelitis virus *in vitro*. Equal parts of the encephalomyelitis virus (20% brain suspension diluted 1 to 25 with saline) and the undiluted serums were mixed, incubated 2 hours at 37°C. and held in the icebox over night. 0.3 cc. of each mixture was then injected intracerebrally into a series of guinea pigs, 2 for each serum. Control animals were also inoculated with the same amounts of normal monkey serum and 1 to 25 virus. Temperatures were taken daily. All of the animals showed a typical rise in temperature followed by symptoms of the disease. Prostration and death occurred after 4 or 5 days, showing a complete absence of neutralization.

The serums of 4 rabbits and of one monkey recovered from encephalomyelitis neutralized the latter virus *in vitro*. Each serum was used in a second series of *in vitro* neutralization tests, prepared with the poliomyelitis virus in the proportion of 1 cc. of serum to 0.3 cc. of 5% virus. The mixtures were kept for 2 hours at 37°C., held over night in the ice box and injected intracerebrally into each of 4 monkeys. A control animal was given normal rabbit serum and virus. All except one of the monkeys became completely prostrated and were killed in 5 to 11 days. One animal showed no definite paralysis but had a high temperature for several days, accompanied by a tremor and preparalytic symptoms. No cross neutralization occurred, therefore, between the encephalomyelitis serum and the poliomyelitis virus.

Three monkeys which recovered from an experimental infection with the poliomyelitis virus were then injected intracerebrally with 1.5 cc. filtrate of a 20% encephalomyelitis virus. A normal monkey and 2 guinea pigs were inoculated at the same time. One of the monkeys had completely recovered from poliomyelitis, one had shown paralysis of an arm with muscular atrophy, while the third had never been able to use both legs and was crippled. The serum of each monkey neutralized the poliomyelitis virus *in vitro*.

All of these animals, including the guinea pigs, showed high temperatures and developed typical symptoms of encephalomyelitis within the usual incubation period. One monkey died and the others were killed after complete prostration.

From these experiments, it seems evident that no cross immunity

occurs between the encephalomyelitic and the poliomyelitic virus diseases although they exhibit certain similarities with respect to epidemiology and pathology. The horse, therefore, need not be considered a possible reservoir for poliomyelitis in man.

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Reaction of the Perfused Isolated Lung of Sensitized Guinea Pig to Horse Serum.

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There has been great need of a simple method of studying objectively the response of the bronchial smooth muscle of the sensitized guinea pig to antigens. Older methods of observing changes in bronchial tone in intact animals are not only difficult of application to small animals but are often complicated by changes in circulation which make the interpretation of such observations uncertain. More recently Koessler and Lewis¹ obtained striking graphs in the sensitized guinea pig by studying the changes in intrapulmonary pressure and the corresponding variations in ventilation depending upon varying degrees of bronchospasm produced by the injection of horse serum into horse serum sensitive guinea pigs. Similar responses may be obtained by perfusing the isolated sensitized lung of the rabbit (Sollmann and von Oettingen²) and of the guinea pig (McDowall and Thornton³). This method is superior to the use of ring preparations of tracheal or bronchial muscle because by means of it a study can be made of the reactivity of the smaller bronchioles which play the major part in bronchial reactions.

This method has been applied by us to a study of the reactivity of the bronchial smooth muscle of guinea pigs sensitized to horse serum. The procedure consists essentially of perfusing the bronchi through the trachea, the fluid making its exit through scarifications on the lung surface. The arrangement of apparatus and the technical details are described by Sollmann and von Oettingen,² and

¹ Koessler, K. K., and Lewis, J. H., *Arch. Int. Med.*, 1927, **39**, 163.

² Sollmann, T., and von Oettingen, W. F., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 692.

³ McDowall, R. J. S., and Thornton, J. W., *J. Physiol.*, 1930, **70**.

Warnant.⁴ We have used the perfusion fluid recommended by McDowall and Thornton. It has the following composition, the amount of each salt being expressed in %: NaCl 0.659, KCl 0.046, CaCl₂ 0.005, MgCl₂ 0.009, NaH₂PO₄ 0.01, Na₂HPO₄ 0.008, NaHCO₃ 0.252. Such a modified Locke's solution is less likely to cause spasm of the bronchi because it contains less calcium and a small percentage of magnesium and its content in buffer salts keeps the hydrogen ion concentration constant for long periods of time.

The rate of flow through the bronchial tree was measured by recording the amount of fluid which filtered from the lung. Alterations of capacity through edema and stretching have been estimated to be negligible in a lung with so small a volume as that of the guinea pig lung. The normal unsensitized lung is only little influenced by the introduction of horse serum into the perfusion system. In some experiments, however, the outflow was reduced about 15 cc. per minute, indicating that some bronchoconstriction may occur in the normal bronchi, due to the slight toxicity of the horse serum and its content in tricesol used to preserve it. We have found that a 0.25% tricesol solution will alone cause bronchospasm, whereas fresh human serum produces no effect.

Contrasted with these responses, which may be regarded as normal, the bronchi of the sensitized animal go into rapid and violent constriction as soon as the injected horse serum reaches them. The amount of fluid which flows through the bronchial tree may fall from 50 or more cc. per minute to a few cc. The addition of 10 or more mg. of epinephrine at this point will not suffice to produce bronchial relaxation.

This reaction appears to be specific for the injection of a 1% solution of crystalline egg albumen before the horse serum is added produces no bronchoconstriction. The method also affords an opportunity of demonstrating the phenomenon of desensitization in the isolated sensitized lung. If, after the bronchi are relaxed following the initial injection of horse serum, a second dose is introduced, no further bronchoconstriction takes place. In both of these respects the perfused isolated lung of the sensitized guinea pig reacts in a similar manner to the excised uterus as shown by Dale in his studies of anaphylaxis.

⁴ Warnant, H., *Arch. internat de pharmacodyn, et de therapie*, 1930, **37**, 61.

Reaction of Bronchial Smooth Muscle in Sensitized Guinea Pigs on a Vitamin C Deficient Diet.

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In a previous communication¹ we gave experimental evidence for the conclusion that removal of vitamin C from the diet of virgin female guinea pigs causes a loss of uterine smooth muscle reactivity to antigens and smooth muscle stimulants. This paper records similar observations on the effect of this dietary deficiency on the sensitized smooth muscle of the bronchi to horse serum, which calls attention to a lack of parallelism existing between the smooth muscle responses in these 2 different regions.

Virgin female guinea pigs weighing 250 to 350 gm. were fed for about 4 weeks on a diet of rolled oats and evaporated milk, a little bran being added to prevent possible intestinal obstruction from the hard fecal masses produced by the oats and milk. When the animals had been on this diet for about a week, they were sensitized by the intraperitoneal injection of 1 cc. horse serum. Some time between the second and third weeks the guinea pigs were sacrificed and simultaneous observations made on the smooth muscle reactivity of the bronchi and uterus of the same animal. The bronchiolar muscle reactions to horse serum were studied by the bronchial perfusion of the isolated, sensitized lung,² whereas the uterine segments were suspended in warmed Locke's solution.

Preliminary results support the conclusion that there is a lack of parallelism in the reactivity of the bronchial and uterine smooth muscle in sensitized animals on a vitamin C deficient diet. Whereas the uterine strips of such animals fail to respond both to antigen and smooth muscle stimulants, histamine, pituitary and barium, the introduction of horse serum and these pharmacologic agents into the bronchial tree cause a marked bronchoconstriction as evidenced by a reduction in the outflow of the perfusion fluid through the lung.

In five out of six guinea pigs on a vitamin C deficient diet the uterine and bronchial smooth muscle responded as recorded in the

¹ Hurwitz, S. H., and Nicholls, E. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **28**, 139.

² Hurwitz, S. H., and Wessels, A. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 120.

paper. Twelve animals were used as controls, in them both the smooth muscle of the bronchi and uterus responded to antigen and smooth muscle stimulants.

These results are in agreement with some unrecorded observations made on anaphylactic shock in sensitized guinea pigs fed on vitamin C deficient diet. In these animals the intracardiac injection of the second dose of horse serum produced typical anaphylactic shock with rigid distention of the lungs. Death usually resulted in 2 to 5 minutes both in these animals and in those on a normal diet.

The results do not support the general assumption that the reactivity of anaphylactic smooth muscle in different regions is essentially the same. A vitamin C deficient diet modifies the responses of the sensitized uterus without affecting the sensitized bronchi. This lack of parallelism is difficult to explain unless it be assumed that the uterine smooth muscle being limited to one sex, is more highly specialized and functionally more readily influenced by dietetic and other factors.

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Device for Automatic Measurement of Oxygen Consumption in Closed Circuit Type of Metabolism Apparatus.

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In the closed circuit type of metabolism apparatus the measurement of oxygen consumption is usually dependent upon changes in the internal pressure of the system. Inasmuch as carbon dioxide is rapidly removed by absorption in alkali, the consumption of oxygen necessarily leads to a reduction in pressure in a system of given volume. The rate of oxygen consumption is then ordinarily determined by measurement of the quantity of oxygen that must be added for restoration of the original pressure, or by compression of the residual gas through the addition of a measured volume of liquid.^{1, 2}

The device now to be described is based upon the latter principle, and permits automatic measurement of the volume of liquid that must be added to maintain a constant pressure within the system. It consists of a 4-liter bottle which serves as an oxygen reservoir,

¹ Wesson, L. G., *J. Nutrition*, 1931, **3**, 503.

² Ebeling, A. H., and Corey, R. B., *J. Exp. Med.*, 1930, **51**, 41.

connected above with the measuring unit proper. This is a 15 cc. cylindrical cup into which water flows from an external feed line as the pressure decreases in the metabolism apparatus. As the cup fills, a float, carrying a light platinum bridge, rises and breaks an electrical contact which is maintained through mercury tubes in the base of the cup. This interruption in the circuit is recorded by a magnetically operated needle on a smoked-paper kymograph. At the same time the cup empties through a syphon attached to the side, the electrical contact is remade, and the water discharged from the cup passes through the syphon outlet into the oxygen reservoir. The displaced oxygen rises through a second tube and enters the space in the upper part of the cup. From here 2 other tubes run. The first, a mere capillary, is connected as a side line with the main circuit of the metabolism apparatus through which air is circulated by a blower. Pressure decreases in the animal chamber are thus directly transmitted through the capillary side line to the cylindrical cup. The second is an external feed line through which water enters and is connected with a source of running water which is maintained, through a simple overflow, at a constant pressure. When the metabolism chamber is empty the pressure head which is maintained in this outer feed line is necessarily equal to the pressure in the main circuit and water does not enter the measuring unit. But during a metabolism experiment the decrease in pressure resulting from the utilization of oxygen causes water to be sucked into the measuring unit in quantities sufficient to compensate for the pressure decrease.

The capacity of the syphon is readily determined by measurement of the average amount of water which passes into the reservoir at each discharge. As used for metabolism experiments with rats we have found 13 or 14 cc. to be a convenient capacity, as it ensures a discharge of the syphon every 2 or 3 minutes.

To guard against fluctuations in pressure due to changes in temperature, the oxygen reservoir and the animal chamber are kept in a water bath at $28.0^{\circ} \pm 0.01^{\circ}$. The metabolism room is maintained at $28.0^{\circ} \pm 0.3^{\circ}$.

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**Comparative Toxicity and Protozoacidal Action of Acetarzone,
Carbarsone, and Certain Related Pentavalent Arsenical
Compounds.***

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We have already shown¹ that 4-carbamino-phenyl-arsonic acid ("carbarsone") is biologically more active than acetarzone, N.N.R., ("stovarsol," 3-acetyl-amino-4-hydroxy-phenyl-arsonic acid). On the basis of oral toxicity in animals and amebacidal concentration *in vitro*, carbarsone has a therapeutic index about 8 times as great as acetarzone. This fact warranted, we believe, a thorough study of carbarsone and related pentavalent arsenicals from the standpoint of their possible application to the treatment of amebiasis. The following compounds were obtained for such an experimental evaluation:

1. $\text{NH}_2\text{CONH} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}_3\text{H}_2$, 4-carbamino-phenyl-arsonic acid,‡ "carbarsone".
2. $\text{NH}_2\text{COCH}_2\text{NH} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}_3\text{HNa}$, Sodium N-phenyl-glycinamide-p-arsonate, "tryparsamide".
2. $\text{NH}_2\text{COCH}_2\text{CH}_2\text{NH} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}_3\text{HNa}$, Sodium phenyl-b-amino-propionamide-p-arsonate,§ "proparsamide".
4. $\text{NH}_2\text{CO} \cdot \text{CH}_3 \cdot \text{CH}_3 \cdot \text{C} \cdot \text{NH} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}_3\text{H}_2$, Phenyl-a-amino-isobutyramide-p-arsonic acid,‡ "isobutarsamide".
5. $\text{NH}_2\text{CO} \cdot \text{CH}_3\text{CH}_2 \cdot \text{CH} \cdot \text{NH} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}_3\text{H}_2$, Phenyl-a-amino-n-butyramide-p-arsonic acid,‡ "n-butarsamide".

In Table I, data are presented on the toxicity of these substances in comparison with acetarzone when given in single doses as a powder in gelatine capsules by mouth. With increase in the number of carbon atoms in the side chain of the carbarsone series there seems to be the usually associated increase in toxicity. It is noteworthy that carbarsone, although having the highest arsenic content, is the least

* Part of an extended cooperative study of the chemotherapy of amebiasis, supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

† Lilly Research Fellow.

¹ Leake, C. D., Koch, D. A., and Anderson, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 717.

‡ Supplied through the courtesy of Dr. H. A. Shonle, Lilly Research Laboratories, Indianapolis.

§ Supplied through the courtesy of Dr. O. H. Kamm, Parke-Davis Research Laboratories, Detroit.

TABLE I.
Oral Toxicity of Certain Arsenical Compounds.

Drug and Actual Arsenic Content of Sample Used		Mortality Ratio at Single Oral Doses in Guinea Pigs	Mortality Ratio at Single Oral Doses in Rabbits	Mortality Ratio at Single Oral Doses in Cats
	%	mg./kg.	mg./kg.	mg./kg.
Sodium Acetarsonic	25.3	4/10 at 100 9/10 " 200	2/4 at 150	5/7 at 150
Carbamino-phenyl-p- arsonic acid	28.3	6/10 " 200 7/10 " 300	4/10 " 200	3/6 " 250
Sodium N-phenyl-glyci- namide-p-arsonate	24.6	7/10 " 150 10/10 " 250	4/5 " 200	1/5 " 200
Sodium phenyl-b-amino- propionamide-p-arso- nate	26.0	6/15 " 100 10/10 " 200	4/6 " 200	3/6 " 200
Phenyl-a-amino-iso- butyramide-p-arsonic acid	26.5	4/10 " 100 7/10 " 150	—	—
Phenyl-a-amino-n- butyramide-p-arsonic acid	24.0	4/10 " 100 8/10 " 200	4/5 at 50	—

toxic of the series. If death occurred it was ordinarily from 3 to 14 days after giving the doses indicated. Post-mortem gross and microscopic examination revealed for these drugs the same general pathological picture which has already been described for acetarsonic poisoning.² No evidence of injury to the optic tract was noted in any of these toxicity studies, although it might be anticipated in the carbarsone series where a substituted amino group occupies a position para to arsenic.³

In Table II one may find certain data on the protozoacidal activity of some of these compounds. The methods used have been previously described.⁴ Proparsamide and n-butarsamide were too restricted in amount to permit satisfactory study. Iso-butarsamide was not available for this phase of the effort. Tryparsamide did not seem to be sufficiently active *in vitro* to warrant extensive study in naturally infested animals.

² Heitzmann, O., *Arch. f. Derm. u. Syph.*, 1926, **152**, 344; Anderson, H. H., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 267.

³ Young, A. G., and Loevenhart, A. S., *J. Pharmacol. Exp. Therap.*, 1924, **23**, 107.

⁴ For estimation of amebacidal concentration *in vitro*: Koch, D. A., *Univ. Calif. Pub. Zool.*, 1926, **29**, 241; Leake, C. D., Koch, D. A., and Anderson, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 717. For estimation of balantidicidal action in naturally infested guinea pigs: David, N. A., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 196. For study of effect in natural monkey amebiasis: Anderson, H. H., and Koch, D. A., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 838.

TABLE II.
Protozoacidal Activity of Certain Pentavalent Arsenical Drugs.

Drug and Actual Arsenic Content of Sample Used	%	Amebacidal Concentration <i>in vitro</i> at 48 hours	Total Balantidicidal Dose on Divided Oral Administration to Guinea Pigs, harboring <i>Balantidium coli</i>	Notes on Effects in Monkeys (<i>M. rhesus</i>) Naturally Infested with <i>E. histolytica</i>
Sodium Acetarsonone	25.3	1:600	75% cleared at 150 mgm./kg. with 50% mortality	Tolerate 400 mgm./kg. at 30 mgm./kg. daily with clearing of stools during administration only
Carbamino-phenyl-p-arsonic acid	28.3	1:4,000	80% cleared at 120 mgm./kg. with 20% mortality	Tolerate 500 mgm./kg. at 100 mgm./kg. daily with clearing of stools through post-treatment observation period of 3 months in 3 animals, but with recurrence within 30 days in 4 animals
Sodium N-phenyl-glycinamide-p-arsonate	24.6	1:1,000	100 mgm./kg. (Sweeney ⁵)	—
Sodium phenyl-b-amino-propionamide-p-arsonate	26.0	1:10,000	—	Tolerate 800 mgm./kg. at 50 mgm./kg. daily with clearing of stools during administration only
Phenyl-a-amino-n-butyramide-p-arsonic acid	24.0	—	60% cleared at 150 mgm./kg. with 40% mortality	—

Comparative evaluation of the protozoacidal properties of the drugs noted is difficult because the data is inadequate for all except acetarsonone and carbarsone. For these 2 drugs it is obvious that carbarsone is the better, not only *in vitro*, but in natural infestations in guinea pigs and monkeys. The latter can not tolerate, without undue gastro-enteric distress, daily doses of acetarsonone in amounts great enough to clear the stools except during the treatment period. But carbarsone can be tolerated by macaques in doses large enough to achieve more lasting success. Tryparsamide requires a stronger concentration for amebacidal action *in vitro* than carbarsone. While proparsamide appears better than carbarsone *in vitro*, it can not be tolerated by naturally infested monkeys in large enough daily doses to be more than temporarily effective. N-butarsamide seems too toxic to be seriously considered.

Summary. Comparative oral toxicity studies on guinea pigs,

⁵ Sweeney, M. A., *Am. J. Hyg.*, 1929, **9**, 544.

rabbits, and cats, and a comparative protozoacidal evaluation on amebae *in vitro* as well as on natural balantidial infestations in guinea pigs and on natural monkey amebiasis indicates that 4-carbamino-phenyl-arsonic acid ("carbarsone") has definite advantages from the standpoint of application to anti-amebic therapy over acetarsone ("stovarsol"), tryparsamide, "proparsamide," "iso-butarsamide," and "n-butarsamide." It is definitely less toxic on oral administration than these compounds and seems generally more protozoacidal in tolerated doses.

5759

**Effects of Ovaries in Various Stages of Activity and of Pregnancy
Upon Adrenalectomized Rats.**

JESSE L. CARR. (Introduced by C. L. Connor.)

From the Department of Pathology, University of California.

According to Stewart and Rogoff,¹ the survival period of adrenalectomized dogs is prolonged by pregnancy. H. A. Stewart² finds this same mechanism exists in the cat, although E. Z. Corey³ concludes that the presence of corpora lutea have not this influence in the pregnant and lactating female. Mulon and Vincent⁴ suggest that the corpus luteum acts as a temporary adrenal cortical body, thus accounting for the increased survival period which does occur apparently in dogs. Herring⁵ on the other hand, points out that the rat liver is 27.5% heavier in the pregnant rat due to glycogen storage, and that this storage accounts for the extended survival period. Harding⁶ adds that protein economy in pregnancy may extend this period.

In the white rat, fresh corpora lutea are constant in the ovary and exist in 3 forms, those of ovulation, pregnancy, and lactation. According to Evans, each of these may have certain characteristics and these may be distinguished both grossly and microscopically. The following experiments are the results of an effort to determine

¹ Stewart and Rogoff, *Am. J. Physiol.*, 1927, **79**, 508.

² Stewart, H. A., reported by MacCallum, *Int. Cong. Med.*, London, Sec. 3, 173.

³ Corey, E. Z., *Phys. Zool.*, 1928, **1**, 147.

⁴ Vincent, S., cited by Britton, "Internal Secretion of the Ductless Glands," (Monograph), London.

⁵ Herring, P. T., *Brit. Med. J.*, 1920, **2**, 886.

⁶ Harding, V. J., *Physiol. Rev.*, 1925, **5**, 279.

the influence of these corpora lutea upon the post-operative survival period of bilateral adrenalectomized white rats.

I. *The influence of ovariectomy.* Eighteen 250 gm. female white rats, each experiencing a normal oestrus cycle, were selected. Six of these were kept under uniform conditions as controls. In the remaining 12, both adrenals were removed and in 6, both ovaries were removed as well. Ether anesthesia was used and all 18 of the animals were subjected to the same period of inhalation. The entire group was kept in an isothermic chamber at 37° and were fed the normal laboratory diet. The animals were observed and their daily weight, temperature, and oestrus cycle recorded.

Group I. Control. Of this group, all the animals either kept their weight within normal fluctuations or gained. They maintained a normal body temperature and sexual rhythm, and their alimentation was normal for the period.

Group II. Bilateral Adrenalectomy. Oestrus was immediately interrupted. The animals lost an average of 5 gm. per day, and the temperature declined from an average of 38.1°C. the day of operation to 35.6°C. the fifth day post-operative. They became asthenic on the fourth day after operation. No diarrhea was observed. One animal died the fifth day, and 4 on the sixth day after surgery, all from adrenal insufficiency. The sixth animal was still living and healthy 22 days later, and autopsy at that time showed a right adrenal remnant left at operation which had doubtless been the factor in survival. The average survival period for the group, eliminating this last member, was 6.4 days.

Group III. Bilateral Adrenalectomy and Oophorectomy. This group lost weight at about the same rate as Group II, 5 gm. per animal per day. The average temperature decreased from 37.3°C. the day of operation to 34.1°C. the seventh day post-operative. The oestrus cycles stopped immediately following operation. Asthenia was noticeable the fourth day, and increased until death. One animal died on the third day with a lung abscess. One died the sixth day. 3 died the eighth day, and one died the eighteenth day after operation, all from adrenal insufficiency. The eighteen-day period must have been supported by an adrenal remnant left at operation, although it could not be found at autopsy. Eliminating these 2 extreme cases, the average survival period for this group was 7.5 days.

Conclusion. Oophorectomy and the absence of the corpora lutea of ovulation does not shorten the survival period of bilateral adrenal-ectomized white rats.

II. *Effect of the presence of the corpus luteum of lactation.*

Of 7 females in this group, 2 were operated upon the day before delivery, one 7 days, one 8 days, one 10 days, one 14 days, and one 16 days post partum. Except in the first two, lactation was well established and adequate. Exploration of the ovaries at the time of operation showed the presence of many large, firm lutein bodies in each ovary. In the first 2 individuals, the breasts were never well filled with milk and the young remained weak until death. In the others, active lactating organs declined and were dry from the second to the fourth day.

The young, normal and active until operation upon the mother, were moribund between the second and fifth days, post-operative, and died from the third to the sixth day after maternal adrenalectomy, the average post-operative survival period of the young being 3.8 days. The mothers passed through the usual stages of decline due to adrenal insufficiency, with weight loss, lowering of body temperature, asthenia, and death, which occurred on the fourth day after operation in 2, the fifth day in one, the seventh in 3, and the ninth in one, with an average of 6.0 days for the series.

Three lactating females, etherized only, showed no alteration in health or milk supply.

Conclusion. The presence of the corpus luteum of lactation does not prolong the life of bilateral adrenalectomized white rats.

III. Effect of pregnancy. Of the 7 animals in this group, one was in the seventh day of gestation, one in the thirteenth, 2 in the fifteenth, one in the sixteenth, one in the seventeenth, and one in the eighteenth day of gestation. Bilateral adrenalectomy was done under the standard conditions outlined. A group of 3 pregnant females were etherized only. No significant changes were noted in these and they went on to normal term delivery.

The 7 animals operated upon showed the usual signs of adrenal insufficiency on the fourth day, with loss of weight and body temperature, asthenia, and death. Three animals died with the young stuck in the birth canal on the third, sixth, and eighth days respectively; a fourth died on the sixth day after aborting, and three died on the fourth, seventh, and tenth days, respectively, with the young apparently viable in the uterus. Autopsy showed a coincident pneumonia in the animal dying on the seventh day. Other autopsy findings in the maternal and foetal tissues will be reported later. The average survival period for this group was 6.3 days.

Conclusion. Pregnancy in the white rat does not prolong the survival period after bilateral adrenalectomy.

5760

Effect of Swingle's Extract upon Lactation in the Adrenalectomized White Rat.

JESSE L. CARR. (Introduced by C. L. Connor.)

From the Department of Pathology, University of California.

Six lactating animals of 200 gm. average weight were included in this group. Three females one day post partum were etherized and kept under normal conditions as controls. One female 4 days, and 2 females 7 days post partum were adrenalectomized. In these mothers the milk supply had developed and the young were thriving. Beginning the day of operation, the 3 animals operated upon received one cc. of Swingle's cortical extract subcutaneously, each cc. being equivalent to 40 gm. of beef cortex. In the control group the milk supply developed normally and the nourishment of the young was without variation from normal limits. In each of the other 3 there was a noticeable decrease in the supply of milk the second day after operation and lactation ceased on the third. The 4-day-old young died on the fourth day after the operation on the mother, and the young 7 days old were moribund on the fourth day, when they were transferred to other mothers for nourishment. The action of this group parallels that of the animals in Experiment II, where the milk supply failed the second to the fourth day with death of the young from the third to the fifth days post partum.

Conclusion. The substance necessary to maintain lactation in the white rat is absent in cortical extract made after the method of Swingle.

5761

Effects of Adrenal Cortical Extracts upon the Sex Organs of Animals.

C. L. CONNOR.

From the Department of Pathology, University of California.

I. *Effect of Swingle extract on maturity in rats.* In view of the known effect of certain adrenal tumors, and possibly hyperplasias, in producing sexual precocity in children, and because of the general impression that mature rats were less susceptible to sarcoma than

younger animals, an attempt was made to produce precocious maturity in rats, with the intention of then testing their relative susceptibility to tumors. One of the methods used was to inject young rats with adrenal cortical extract, prepared after the manner of Swingle and Pfiffner.¹ Four litters of rats were used, half of which were injected daily with extract in doses amounting to 20 gm. of adrenal cortex to 200 gm. of rat weight. The extract was freshly prepared every 10 to 12 days, and was tested on adrenalectomized rats to insure its potency.

Corey and Britton² have reported changes in the ovaries and testes of rats following similar treatment, which they interpreted as a stimulation to sexual maturity. The present groups were watched carefully for signs of maturity (the opening of the vagina and vaginal smears in the females; the behavior of the males in the presence of females in oestrus), and it was found that the controls matured as early, and in some cases earlier, than the injected animals. (Table I.)

TABLE I.

Litter	Age at Start	Weight at Start average	No. Injections average	Weight at Maturity average	Age at Maturity
	Rats	Days	gm.	gm.	Days
T	3	19	17.2	89	90
C	3	19	17.0	—	92
T	4	40	25.0	93	85.2
C	3	40	29.6	—	117.0
T	4	60	51.6	44	111.3
C	3	60	63.3	—	125.0
T	4	80	107.0	36	141.2
C	4	80	119.5	—	149.2
Treated	15				118.0
Controls	13				124.0

T = Treated; C = Controls.

II. *Effect of Swingle extract on egg-laying in chickens.* In May, 1930, I reported a degeneration of the ovaries of chickens following the injection of a watery extract of adrenal cortex.³ It was desirable to know whether Swingle and Pfiffner's lipid extract acted in the same way. Five laying hens were injected every other day with 1 cc. of Swingle extract. These chickens were followed for 49 days, receiving 25 injections. The hens continued to lay regularly, producing on an average of 21 eggs in this period. This is in marked contrast to the experiment reported in which watery extract

¹ Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153, 164.

² Corey, E. L., and Britton, S. W., *Science*, 1931, **74**, 101.

³ Connor, C. L., *Arch. Path.*, 1930, **9**, 1296.

was used. This quite constantly produced degeneration of the ovaries with immediate cessation of ovulation.

III. *Effect of various extracts on the oestrus cycle in rats.* Watery extracts of adrenal cortex were injected in comparatively large doses into 13 female rats which were having oestrus cycles at regular intervals. From 10 to 15 daily injections of 2 cc. each were given. This was made by macerating adrenal cortex from beeves in an equal amount of Ringer's solution and filtering the liquid through a Seitz filter. It was always used fresh. With the exception of one rat, this treatment caused the oestrus cycles to cease. In this one rat (Rat 4, Table II), the dioestrus period was considerably prolonged. In 6 rats (8, 9, 10, 11, 12, 13) injections were stopped,

TABLE II.
The Oestrus Cycle of Rats Following the Injection of Various Substances.

A. Ringer's solution extract of adrenal cortex, 2.0 cc. daily, 10-15 days.	
Rat	
1.	-- 000 --- 00 --- 00 --- oIo ----- C-----
2.	-- 00 --- 000 --- 00 --- 00I ----- C----- 00 --- 00
3.	00 --- 00 - Io0 --- 00 --- C-----
4.	-- 00 --- oI --- 00 --- 0 --- C-- 0 --- 00 --- 00
5.	0 --- 00 - I - 00 --- C----- 00 ---
6.	--- 00 --- I - 00 --- C----- 00 ---
7.	--- 00 --- Io0 --- C-----
8.	--- Io0 --- C----- oIo --- 00 --- C-----
9.	--- oIo --- C----- oIo --- C----- 0
10.	--- I --- C----- oIo --- 00 --- C----- 0
11.	000I --- C----- oIo --- C-- 0 ---
12.	oI --- 00 --- C----- oI --- C----- 00 ---
13.	- Io0 --- 00 - C----- oIo --- 00 --- C----- 00 ---
14.	- 00 --- IC0 - 00 --- 00 --- 00 --- 0
15.	- 00 --- 00 - IC - 00 --- 00 --- 000
16.	- 00 --- 00IC - 00 --- 00 --- 000
17.	00 --- 00 --- IC00 --- 000 --- 000
B. Protein (veal broth), 2.0 cc. daily for nine days.	
18.	-- 00 --- Io0 --- 00 - C-- 00 --- 00
C. Adrenalin (1.0 cc., 1-100,000 sol. 5 days; 1-50,000, 7 days).	
19.	00 --- 00I --- 00X ----- oC
20.	--- 00 - I --- X000 --- oC
21.	--- 00I --- X --- 00C
22.	-- 00 --- I --- X ----- C
23.	-- 00 --- I --- 00X - 00 --- oC
24.	-- 00 --- I - 000X - 00 --- C
25.	-- 00 --- I --- X ----- (died, pneumonia)
26.	--- 000 - I - 00 - X - 000 --- C
27.	--- 00 --- I - 000X - 000 --- C
Swingle Extract (1 cc. daily for 7 doses)	
28.	- 00 --- 00 - I - 00 --- C00 --- 00 ---
29.	- 00 --- 000I --- 00 --- C - 000 --- 000
30.	--- 000 --- I - 00 --- C - 00 --- 000
31.	--- 00 --- I - 00 --- 00 --- 00 ---

In the above table, o means oestrus, dash (-) means dioestrus; I means injections started, C means injections ceased; X indicates when the dose of adrenalin was doubled; IC—only one injection was given to this group.

and after an average interval of 13 days, the cycle was resumed. Further injections caused a second cessation of oestrus which persisted until from 4 to 10 days after the last injection. Four rats (14, 15, 16, 17) were given only 1 dose (2 cc.) and no effect was produced. Rat 18 was injected repeatedly with 2 cc. of veal broth without affecting its cycle. Nine rats (19, 20, 21, 22, 23, 24, 25, 26, 27) were given 5 injections of 1 cc. each of a 1-100,000 solution of adrenalin. Then the dose was doubled for 8 daily injections. This caused no effect in 6 rats. In one, oestrus did not take place. Another rat had no cycle. This one died on the 13th day of pneumonia. Four rats (28, 29, 30, 31) were injected daily for 7 days with 1 cc. (representing 30 gm. of adrenal cortex) of an extract made after Swingle's method. This caused no change in the sex cycle.

From these experiments we can conclude that the adrenal cortex contains a substance which inhibits the activity of the gonads of mature animals and that, while the lipoid extract of Swingle contains the principle necessary to maintain life, it does not contain the sex-affecting portion of the adrenal gland.

5762

A Method for Studying the Microscopic Structure of Bacterial Colonies.

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The study of bacterial colonies has in recent years been stimulated by investigations of microbic dissociation. The existence of variant strains, variously developed, which differ in pathogenicity, in biochemical reactions, in antigenic structure, and in colonial characteristics can no longer be doubted. Furthermore, almost no attempts have been made to explain the mechanism whereby bacterial cells pile up to produce colonies varying widely in gross morphology. The relation of pleomorphic bacteria, and of organisms having abnormal staining characteristics, to the development of cells into masses containing tremendous numbers and representing a considerable number of generations is largely unknown. Technical difficulties are responsible for many of these omissions. The ordinary

smear is crude. The impression colony is clumsy, and it is limited to certain types of very small colonies. Legroux and Magrou¹ described a method for making paraffin sections of old colonies of *V. cholerae*, but they found that their method failed with surface colonies because they were washed off by the solutions used. The method to be described overcomes these difficulties, and may be applied to any type of bacterial or mold colony.

Colonies on or in agar medium are used. For surface colonies the medium is warmed to 45°C., and over it is gently flowed 1% agar, melted and cooled to 60°C. Thus a protective layer is formed over the colonies without distortion, the upper layer adhering to the lower. Mold colonies should first be wet with 80% alcohol to prevent the formation of bubbles. When the agar hardens the desired section is cut out and dropped into the fixative. Deep colonies need no preliminary handling. Both 10% formalin and Zenker's solution have been used, with 12 to 24 hours' exposure, with equally good results. From this point the block of agar is treated as any delicate piece of tissue by the usual histologic technic of dehydrating, clearing and embedding. Great care must, however, be used in removing all water by frequent changes of absolute alcohol and in removing all alcohol. Otherwise the agar shrinks and hopelessly distorts the colony, and it becomes rubbery when embedded. In order to bring out individual cells sections must, of course, be cut very thin. With good preparations sections 1 to 3 micra in thickness are possible. Mold colonies, being less compact, are satisfactory in sections of 5 to 7 micra. Sections are spread and mounted in the usual way, and when firmly fixed on the slide they are put through xylol and the alcohols to water. Then any of the usual stains may be applied. The agar does not take a definite stain, but if eosin is put in the dehydrating alcohols a pink background will be obtained. Only a few of the many stains which suggest themselves in this technic have been tried. After staining, the section is mounted in balsam.

The only observations which have been made, using this technic, have been those noted incidentally in the development of the method. Sections of mold colonies reveal diagonal and transverse sections of hyphae and possibly of spores which may be of cytologic interest. Sections of *B. subtilis* colonies, cut transversely, reveal suggestive patches of Gram negative cells on the colony surface, as well as some tendency to pleomorphism on the same surface. A cross section of *Bact. paratyphosum* B, using a rough variant colony, gave a picture of a very densely packed mass of cells, with relatively wide

¹ Legroux, R., and Magrou, L., *Annales de l'Inst. Pasteur*, 1920, **34**, 317.

bands, the significance of which has not been determined. A deep colony of anaerobic species, *Vibrio septique*, in contrast presented a loose or mesh structure composed of filaments of cells bent in an interesting fashion.

5763

Change of Dexterity with Age.*

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It is a common impression that old age is accompanied by a slowing down of the voluntary processes both muscular and mental. Industrial experience and every-day observation of older adults seem clearly to indicate this change. When decrement begins, the rapidity with which it proceeds and the amount of slowing generally to be expected by the chronological ages of 60 or 70 years, are all, at present, largely matters of personal opinion. The developmental increase, for example, in motor facility from early childhood through adolescence and into adulthood, has been studied extensively. But adults in middle age and in the period of later maturity have not been conveniently available for laboratory investigations and moreover, they seem to have been actually neglected as subject material for psychological experimentation.

For the present study, a "laboratory annex" was set up in a small city and people were secured for experiment and measurement through the coöperation and solicitation of clubs, lodges, churches, and many similar agencies. An individual who came to the annex for the 2-hour series of tests was not paid but the club that sent him was paid proportionate to the person's age. The person himself knew just how much he was contributing to this club. The study includes 166 men over 50 years of age, and 169 males, younger; 302 women over 50 years and 226 women and girls younger than 50.

The total period of measurement was 2 hours in length and included several short tests and experiments.^{1, 2} The present report

* Part of a study on Later Maturity conducted in the Psychology Department of Stanford University, and made possible through a grant from the Carnegie Corporation of New York.

¹ Miles, W. R., *J. of Psychol.*, 1931, **43**, 377.

² Miles, C. C., and Miles, W. R., *Am. J. Psychol.*, 1932, in press.

deals briefly with only one of these which came in the first half-hour period, a test of speed and accuracy in reaching and grasping. Two small blocks of wood were located 12 inches apart on a thin panel or lap board. Two holes, $1\frac{1}{2}$ inches apart, were made in each block and the 4 holes were in one line. A small electric clock (operating on 60 cycle A.C.) was placed midway between the blocks so that the button or key controlling the clock was on the line made by the 4 holes. Each hole was $1\frac{1}{2}$ inches deep and $\frac{3}{8}$ inch in diameter. For each block there was a piece of ordinary round lead pencil 3 inches long which was placed in one of the holes. The distance from the key to the nearer hole of each block was 6 inches. The subject sat with the panel directly in front of him on a table. With his right fore-finger he pressed against the clock key and when ready to make a trial reached directly to the right and with his right hand grasped the pencil, thrust it in the other hole of the same block and returned his fore-finger to the key. This was done as deftly and as quickly as possible. The clock hand started and the clock movement began a buzzing sound as soon as he moved his hand in the direction of the pencil. The completion of the act and the return of his finger to the key stopped the clock. The time consumed was evident to both subject and examiner. When this value had been read off and written down the left fore-finger was substituted for the right and the coördination was tried with the left hand reaching for the pencil in the block at the left. Each hand was tried three times alternately. This coördination was, of course, voluntary and self-initiated and did not in any way depend upon signals from the examiner. Each subject, by performing the act, measured his own time. In instructing the subject no demonstration or sample performance was given, he did not imitate another person's action, but was asked to be quick. Motivation was good, since subjects could easily understand the situation and could see their own results.

In this test slowness of movement and inaccuracy both serve to increase the time. In general, adults require about 1.2 seconds to accomplish this coördination with the right or dominant hand and 1.4 seconds for the left or subordinate hand. The range shown by individuals from early to late adulthood is around 0.9 to 5.0 seconds. The correlation between the performance with the dominant hand and that with the subordinate one is approximately $r + 0.53 \pm 0.05$.

The results arranged by semi-decades, and with 2-year steps for the early ages, are presented in Table I. This shows the number

TABLE I.
Age changes of dexterity in reaching and placing.

Ages	Males			Females		
	No. cases	Dom. Hand (Sec.)	Sub. Hand (Sec.)	No. cases	Dom. Hand (Sec.)	Sub. Hand (Sec.)
90-94				2	3.96	5.10
85-89	2	1.81	1.59	4	1.91	2.07
80-84	11	1.85	1.93	14	1.81	1.98
75-79	15	1.58	1.78	23	1.54	1.78
70-74	27	1.39	1.56	41	1.64	1.82
65-69	27	1.41	1.40	54	1.44	1.64
60-64	26	1.35	1.40	60	1.39	1.55
55-59	33	1.24	1.40	53	1.32	1.44
50-54	25	1.17	1.35	51	1.27	1.45
45-49	18	1.12	1.33	30	1.24	1.38
40-44	21	1.15	1.34	27	1.12	1.32
35-39	20	1.16	1.29	22	1.13	1.30
30-34	20	1.14	1.34	28	1.14	1.28
25-29	19	1.12	1.22	17	1.10	1.36
20-24	23	1.11	1.28	21	1.12	1.34
18-19	7	1.19	1.26	10	1.14	1.38
16-17	11	1.10	1.19	13	1.19	1.32
14-15	6	1.15	1.44	21	1.28	1.40
12-13	8	1.26	1.48	22	1.20	1.46
10-11	6	1.29	1.52	7	1.28	1.60
8-9	8	1.37	1.65	8	1.45	1.51
6-7	2	1.69	1.56			
Total	335			528		

of cases examined (males and females) at the different age levels, and gives the average dexterity time for dominant and subordinate hands separately. It will be observed that there is no sharp high peak of dexterity. The best performance falls between the ages of 16-29 where the average for both hands and both sexes is close to 1.2 seconds (dom. 1.15, sub. 1.30 sec.). Groups younger or older require more time. For 15 years above age 29 there is but slight decrement. If we take 1.2 seconds for base, then 55-59 shows 14% increase; 60-64, 18%; 70-74, 33%; and 85-89, 54%; while ages 8-9 show 25% increase.

There is a remarkably close agreement in the results for males and females up to the age of 45 years, beyond this the men score a little faster. The most striking fact in the whole set of results is the relatively small, although steady, decrement shown throughout the life span proceeding in both directions from age 20. It might easily have been guessed that people 80 years of age would require double time for such an act. That they require on the average only 50% longer than so-called prime adults, is significant of the continuity in the powers of man's nervous system.

5764

Experimental Observations on the Anesthetic Properties of Divinyl Ether.*

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Studies have been reported on the anesthetic properties of certain unsaturated ethers,¹ from which it seems that divinyl ether is a compound deserving thorough investigation as a potentially useful general anesthetic agent. This earlier survey was made on an apparently impure sample (B.P. 36-39°C.). Thanks to Ruigh and Major² an adequate supply of pure divinyl ether (B.P. 28.2-28.5°C., corrected; Sp. Gr. 0.77 at 20°C.) is now available, and it is a pleasure to acknowledge their kindness in furnishing the substance for our present use.

The range of minimal anesthetic concentration of divinyl ether was found to be between 1.35 and 1.75 millimols per liter of air. This was estimated by inhalation for 10 minutes in a series of 104 mice by a method essentially the same as that described by Johnston.³ The time period required to anesthetize 60 different mice at the minimal certain anesthetic concentration (1.75 millimols per liter of air) varied between 30 and 70 seconds. This is more rapid on the average than the time range required to anesthetize this same group of mice with the minimal certain anesthetic concentration of chloroform (0.3 millimols per liter of air). During anesthetization with divinyl ether, mice show a short excitement period, rapidly become ataxic, and may exhibit running movements during the initial period of anesthesia. Upon removal from the anesthetizing chamber, the mice recover at the concentration noted, within 40 seconds. The range of minimal lethal concentration of divinyl ether for mice is apparently above 6 millimols per liter of air on 10 minutes inhalation.

In semi-quantitative experiments in dogs, divinyl ether in oxygen anesthetizes much more rapidly than di-ethyl ether in oxygen, and without as much excitement or struggling during induction. There

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† National Research Council Fellow.

¹ Leake, C. D., and Chen, M. Y., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 151.

² Ruigh, W. L., and Major, R. T., *J. Am. Chem. Soc.*, 1931, **53**, 2662.

³ Johnston, J. F. A., *J. Pharmacol. Exp. Therap.*, 1931, **43**, 85.

is not as much evidence of local irritation with divinyl ether as with either chloroform or di-ethyl ether. It stimulates respiration during induction and rapidly depresses it as deep anesthesia is reached. Cardiac action is not greatly altered even in relatively deep anesthesia. Although the blood pressure falls as anesthesia deepens it is maintained at more than half the normal level even after the anesthetic concentration has been high enough to stop respiration for ten minutes. Upon withdrawing the divinyl ether and oxygen anesthesia under these conditions respiration spontaneously recovers. Divinyl ether does not seem to cause the marked disturbance of the acid-base equilibrium characteristic of di-ethyl ether or chloroform. Recovery of dogs from prolonged anesthetization with divinyl ether and oxygen is more rapid than with either ether or chloroform.

Divinyl ether is as inflammable as di-ethyl ether, and probably as explosive. It is roughly thrice as efficient an anesthetic as di-ethyl ether in regard to concentration in oxygen required to anesthetize, in rapidity of anesthetization, and in promptness of recovery. It is less active than chloroform from the standpoint of concentration in oxygen required to anesthetize, but it is about as prompt in causing anesthesia, and it produces a comparable relaxation. Divinyl ether appears to be preferable either to chloroform or ether from the standpoint of its physiological and pathological effects.

Western New York Section.

Cornell University, October 17, 1931.

5765

Effect of Cortin on the Nervous System in Adrenal Insufficiency.*

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The outstanding symptom in adrenal insufficiency is asthenia. It has been shown experimentally by Gans and Miley,¹ who used a nerve muscle preparation, that only 1/16th of the work done in normal rats could be performed in adrenalectomized rats. They did not show whether the seat of this fatigue was in the myoneural junction or in the muscle itself. Certain clinical observations which we have made recently indicate that the nervous system, even the higher centers, may be involved in the fatigue of adrenal insufficiency.

In the present investigation, we have studied the effect of cortin on the reflex as well as the peripheral structures. Albino rats of the same strain were adrenalectomized and kept under identical conditions. These were divided into 2 series and matched according to weight and sex. The first series was injected twice daily with ½ cc. of cortical extract (40 gm. per cc.), while the other series was injected at the same times with the same volumes of isotonic saline. Beginning 2 days after operation the resistance to fatigue was compared with animals from the 2 series. Amytal was used for anesthesia. The spinal cord was cut just below the diaphragm. Electrodes were placed in contact with the central portion of the sciatic nerve on the right side after it had been dissected free and cut. A second pair of electrodes was placed in contact with the sciatic on the left side. The tendon of the left gastrocnemius was attached to a muscle lever fastened to a scale pan which was after-loaded with a 100 gm. weight. This muscle was pre-

* Aided by a grant from the Ella Sachs Plotz Foundation.

¹ Gans and Miley, *Am. J. Physiol.*, 1927, **82**, 1.

pared for direct stimulation by wires fastened to the tendon and to the origin of the muscle. Care was taken to prevent drying of the nerves and muscle. Supermaximal stimuli were used in every instance. An automatic interrupter which completed the primary circuit, about 3 times a second furnished the stimulus. Both make and break shocks were used. The right sciatic was stimulated until the left gastrocnemius failed to give further contractions, upon which the stimulus was immediately switched to the right sciatic. When the left gastrocnemius again failed to respond, the muscle was directly stimulated. This arrangement enabled us to determine the fatigue time of the reflex arc, myoneural junction and the muscle itself.

The average times of completed tests are given in Table I.

TABLE I. *Average Time Required for Fatigue.*

Series	No. of cases	Reflex (min.)	Myoneural Junction (min.)	Muscle (min.)
Cortin	10	124	234	255
NaCl	9	19	37	43

The difference in the 2 series is significant, since the cortin series required 6 times as long to fatigue in every instance.

There is evidence that more frequent injections of cortin will increase the difference between the 2 series, but the number of animals used is too small to draw any further conclusions.

5766

The Recovery Oxygen Consumption of Muscles Poisoned with Brom-acetic Acid.

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Lundsgaard¹ reported that the resting oxygen consumption of the intact frog was unimpaired by brom-acetic acid poisoning while glycolysis was completely inhibited. Fischer² using electrical stimulation was unable to find a recovery heat production in isolated frog muscle poisoned with 0.05 to 0.1% brom-acetic acid. Heg-

¹ Lundsgaard, E., *Biochem. Z.*, 1930, **227**, 51.

² Fischer, E., *Pflüger's Arch.*, 1931, **226**, 500.

nauer³ stated that 0.01% brom-acetic acid completely inhibited the lactic acid formation which usually followed KCl stimulation but had no effect on the excess oxygen consumption. In view of the apparent contradiction between the work of Fischer and of Hegnauer a considerable series of experiments was run to determine the excess oxygen consumption and lactic acid production of muscles treated with brom-acetic acid in several concentrations up to 0.2% and then stimulated electrically or chemically. While this work was in progress Hill and collaborators⁴ reported a normal recovery heat production in muscles poisoned with 0.004% iodo-acetic acid.

Sartorius muscles were soaked in Ringer's solution containing brom-acetic acid or its sodium salt for half an hour at a temperature of 8°C. They then were placed in a differential volumeter, a resting rate determined ($\frac{1}{2}$ to 1 hour), stimulated, and the excess oxygen consumption measured. Chemical stimulation was accomplished by dumping 0.9% KCl from a side arm of the experimental bottle. The tension developed by matched muscles similarly treated was recorded on an isometric lever. The excess oxygen consumption and the tension development of electrically stimulated muscles were measured simultaneously on the same muscle. This was accomplished by leading a fine wire from the muscle through a mercury-sealed capillary to an isometric lever. Another respirometer without this mercury seal and containing matched muscles acted as a control on the oxygen consumption. A. H. Hegnauer, to whom I am greatly indebted for his collaboration, made lactic acid determinations on muscles treated and stimulated in an exactly similar manner.

Brom-acetic acid or its sodium salt in concentrations above 0.02% completely inhibited the lactic acid production that usually results from electrical or chemical stimulation.

Forty-four experiments using chemical stimulation at 22°C. showed that there was a definite decrease in excess oxygen consumption as the concentration of brom-acetic acid increased. The tension developed likewise fell off with increased poisoning. Both tension and excess oxygen fell to zero at about 0.08% brom-acetic acid. Whenever tension was developed there was an excess oxygen consumption but the muscles became rapidly non-irritable at this temperature. Fourteen experiments on muscles chemically stimulated at

³ Hegnauer, A. H., *J. Pharm. and Exp. Therapeutics*, 1931, **42**, 99.

⁴ Cattell, Feng, Hartree, Hill and Parkinson, *Proc. Roy. Soc., B.*, 1931, **108**, 279.

14°C. showed an excess oxygen consumption 70% of normal when treated with brom-acetic acid of 0.04 to 0.2%. At this temperature the muscles retained their irritability for several hours after poisoning.

Normal muscles electrically stimulated for 5 sec. at 14°C. gave an average value (6 determinations) of 0.0035 for the quotient

$$\frac{\text{O}_2 \text{ mm}^3}{\text{Tension (gm.)} \times \text{length (cm.)} \times \text{time (sec.)}}$$

where the oxygen represents the excess consumption by the weight of muscle used. Muscles poisoned $\frac{1}{2}$ hour with brom-acetic acid in concentrations of 0.01 to 0.2% gave an average value of 0.0024 for this quotient or approximately 70% of the normal value.

5767

Influence of Carbon Dioxide upon the Respiration of Nerve.*

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The influence of carbon dioxide upon the respiration of the lateral line nerve of the smooth and spiny dogfish has been studied by the tonometric method. Fifteen cc. tonometers which contained besides the nerves one-tenth of a cc. of isotonic solution were placed in a water bath, the temperature of which varied from 20.5 to 23.7°C. in the different experiments. The gas mixture present in the tonometers was analyzed at the beginning and at the end of each experiment on a modified Haldane-Henderson analyzer. The measurement was begun 2 to 3 hours after the removal of the nerves and lasted for 4 or 5 hours. Eight or more hours after the isolation of dogfish nerves the rate of oxygen consumption rapidly increases.

The tonometric method has been checked by burning ethyl alcohol in the tonometers and determining the respiratory quotient. These quotients vary from 0.630 to 0.678 with an average of 0.655.

Ten control experiments showed that the maximum difference in the rate of respiration of matched nerves was 16%. Forty-five experiments were carried out on nerves in air-oxygen gas mixtures. The rate of oxygen consumption varied from 25.1 to 157.0 cu. mm. per gm. of fresh weight per hour, depending upon the size of the

* Aided by a grant from the Hendricks Research Fund, Syracuse University.

nerve. The average was 64.1 cu. mm. The rate of carbon dioxide production fluctuated between 17.3 and 176.0 cu. mm. per gm. per hour with an average of 59.2. The apparent respiratory quotients averaged 0.92.

Calculation of the oxygen tension necessary to supply these nerves by Fenn's¹ or Gerard's² formula indicated that in all cases sufficient oxygen was present to prevent asphyxia. This has been tested directly in a few experiments.

The influence of carbon dioxide upon the respiration of one of a pair of matched nerves has been studied in 24 experiments. The rate of oxygen consumption decreases in approximately a linear manner to 50% of the control value in the presence of 150 mm. Hg carbon dioxide tension. Above 150 mm. Hg carbon dioxide tension, the rate of oxygen consumption decreases more slowly to about 40% of the control at 300 mm. Hg. The rate of carbon dioxide production is depressed to a slightly greater extent than the rate of oxygen consumption. Above 150 mm. Hg carbon dioxide tension the rate of carbon dioxide production was not constant although in 4 of 5 experiments a depression was present.

5768

Excretion of Xylose.

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The constancy of the rate of disappearance of xylose from the blood of animals¹ and the change in the rate of elimination after experimental damage to the kidney membrane² suggested the possibility of its use as a delicate indicator of damaged renal function. The present availability of xylose would render it an excellent substance for kidney function tests in analogy to the liver function tests which employ such sugars as galactose, levulose, etc. Xylose is particularly suited for this purpose since its elimination is practically independent of the liver.

On the oral administration of 50 gm. of xylose, $25 \pm 5\%$ is elim-

¹ Fenn, W. O., *J. Gen. Physiol.*, 1926, **10**, 767.

² Gerard, R. W., *Am. J. Physiol.*, 1927, **82**, 381.

¹ Fishberg, E. H., *J. Biol. Chem.*, 1930, **86**, 665.

² Fishberg, E. H., and Dolin, B. T., *Arch. Int. Med.*, 1930, **40**, 321.

TABLE I.
Normal excretion after oral administration of 50 gm. of xylose.

Time (Hours)	Volume (cc.)	% Reducing Substance	Gm. Reducing Substance
1	36.5	1.7	0.6
2	41.0	3.2	1.3
4	102.0	4.5	4.6
6	172.0	2.9	5.0
8	98.5	1.8	1.8
11	60.0	1.3	0.8
24	151.0	0.2	0.3
Total	661.0		14.4

inated by a normal man within 24 hours (Table I) in the urine. The normal kidney can concentrate this substance up to at least 25% within 2 hours. In the blood the non-fermentable reducing substance rises to 80 mg. % within 3 hours, and after 6 hours the normal value of 28 mg. % is approached. (Table II.) These determinations were carried out by the method of Somogyi.³

TABLE II.
Excretion of xylose in nephritic patient.

Time (Hours)	Volume (cc.)	% Reducing Substance	Gm. Reducing Substance
1	65.0	Neg.	Neg.
2	37.0	0.1	0.04
5	46.0	0.2	0.10
8	40.0	0.15	0.06
11	115.0	0.3	0.3
24	325.0	0.4	1.3
Total	628.0		1.80

TABLE III.
Concentration of non-fermentable reducing sugar in blood.

Time Hr.	Normal Non-ferm. Red. Subst. Mg./%	Nephritic Non-ferm. Red. Subst. Mg./%
0	28	29
1.5	61	80
3.0	80	114
5.0	42	136

On the other hand, in cases of kidney damage the non-fermentable reducing substance in the blood keeps on rising, so that after 6 hours there may be 125 or more mg. % of non-fermentable reducing substance in the blood. The elimination in the urine is much lower, so that figures of 1 to 2 gm. total excretion in 24 hours instead of the

³ Somogyi, M., *J. Biol. Chem.*, 1927, **75**, 33.

normal 12-13 gm. are encountered. The inability of the damaged kidney to concentrate the xylose is particularly striking, so that in cases of severe impairment of renal function, instead of the normal 3% concentration of reducing substance in the urine after 2 hours, we find 0.10% or even less.

New York Section.

New York Academy of Medicine, November 18, 1931.

5769

Radiotherapy and Experimental Herpes Encephalitis in Rabbits.

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The interesting results obtained with radiotherapy in experimental syphilis by Carpenter and Boak¹ have been further extended to the treatment of general paralysis by Hinsie and Carpenter.² These investigations suggested the possible application of radiotherapy to the treatment of experimental virus diseases in animals. Jungeblut and Kopeloff³ inoculated monkeys with poliomyelitis virus but failed to protect the animals with radiotherapy by 5 days treatment for 1½ hours, which resulted in a rectal temperature of 106° to 107°F.

In the foregoing investigation an attempt was made to study the influence of radiotherapy on experimental herpes encephalitis in rabbits. Through the kindness of Dr. W. R. Whitney of the General Electric Company who very generously placed a radiotherm at our disposal and with the assistance of Miss K. King we were able to continue these efforts.

The first rabbit was injected in the *foramen magnum* with 0.2 cc. of supernatant fluid from a 1:20 suspension of a 3-4 day herpes virus (El-l'Perdrau) rabbit brain. This animal was placed in the radiotherm for about 1½ hours on 3 successive days attaining an average rectal temperature of 106-107°F. The control rabbit died with typical symptoms in 4 days. The treated rabbit died 24 hours later. Two rabbits were similarly inoculated with the "Frank" 7

¹ Carpenter, C. M., and Boak, R. A., *Am. J. Syph.*, 1930, **14**, 346.

² Hinsie, L. E., and Carpenter, C. M., *Psychiatric Quart.*, 1931, **5**, 215.

³ Jungeblut, C. W., and Kopeloff, N., *J. Inf. Dis.*, in press.

day herpes virus. When treated by radiotherm their rectal temperatures reached 110°F. and both animals died during the night in consequence of overheating.

Two more rabbits were again inoculated with the "Frank" 7 day virus and these received 7 hours of radiothermy daily for 6 successive days. The average rectal temperature was 108°F. One rabbit reached a temperature of 110.2°F. and died, the other died with typical symptoms 7 days after inoculation as did the 2 control animals similarly inoculated.

Since a temperature of 108°F. failed to protect the rabbits and 110°F. killed them it was of interest to determine the results of heat *in vitro*. We found nothing in the literature to indicate that a critical temperature of 109.4°F. (43°C.) had ever been employed with herpes virus. Consequently, the El-1 strain was heated at 109.4°F. for ½ hour and 7 hours respectively which corresponded to the duration of one radiotherm treatment. Each heated virus material was injected in duplicate rabbits and all 4 died within 5 to 6 days, including the control animal which died in 3 days. These results corroborate those obtained with radiothermy.

Within the limitations of the material at hand it appears that radiothermy in the dosage employed fails to protect rabbits against herpes virus.

5770

Further Observations on the Transformation of Type-Specific Pneumococci by In Vitro Procedures.

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Dawson and Sia^{1, 2} described a method for inducing transformation of pneumococcal types by an *in vitro* technique. In this procedure the transformation of pneumococci from one specific S type into other specific S types was effected through the intermediate stage of the R form. The method consisted in growing small inocula of R forms, derived from S forms of one specific type, in media containing vaccines prepared from cultures of heterologous S types.

¹ Dawson, M. H., and Sia, R. H. P., *J. Exp. Med.*, 1931, **54**, 681.

² Sia, R. H. P., and Dawson, M. H., *J. Exp. Med.*, 1931, **54**, 701.

Under these conditions the R forms frequently were transformed into S forms of the same type as the vaccine. In the course of this study it was observed that the nature of the R culture selected materially affected the results obtained. Thus R cultures which were only partially stabilized in the R form were more readily transformed into S forms of heterologous types than were R cultures which were more definitely stabilized in the R form. This observation suggested the possibility that, under certain circumstances, S forms of one specific type might be directly transformed into S forms of the other specific types.

To insure purity of the strains selected single-cell cultures of a Type II S pneumococcus were obtained.* The virulence of the strains employed was such that 1 cc. of a 10^{-8} dilution of a 15-hour broth culture was invariably fatal for white mice. Small seedings (one drop of a 10^{-6} dilution) of this single-cell II S culture were inoculated into media containing homologous anti-serum together with large quantities of vaccine prepared from a Type III S culture. Control measures adopted to insure the sterility of the vaccines were similar to those described in previous communications.^{1, 3} The cultures were incubated at 37°C. and blood agar plates were streaked at daily intervals for 4 days. In the majority of instances numerous typical III S colonies appeared on plates streaked from cultures which had been incubated for 48 hours. The remaining colonies on such plates were all of the II S variety and no R colonies were observed. These experiments demonstrated that type-specific S pneumococci could be transformed from one specific S type into other specific S types by growth in homologous immune serum in the presence of heterologous S vaccine. Although it was not possible to demonstrate the occurrence of intermediate R forms during the transformation the conditions of the experiment suggest the probability that the transformed organisms passed through a phase comparable to the R state during the transformation process.

Experiments were next arranged to determine the effect of seeding small inocula of S pneumococci in media containing heterologous anti-serum as well as vaccines prepared from heterologous S cultures. One drop of a 10^{-7} dilution of a single cell II S culture was inoculated in media containing Type I serum and III S vaccine. As in preceding experiments the cultures were incubated at 37°C. and

* We are indebted to Mrs. M. S. Ingalls of the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, for assistance in obtaining single-cell cultures.

³ Dawson, M. H., *J. Exp. Med.*, 1930, **51**, 99, 123.

plates were streaked at daily intervals for 4 days. Under these conditions, in occasional instances, it was possible to obtain typical III S colonies on plates streaked from cultures which had been incubated for 24 hours. In these experiments it was again impossible to demonstrate the occurrence of R forms during any phase of the transformation process. Furthermore the conditions provided were those most unfavorable for the development of R forms. However it is not possible to affirm that during the transformation the S organisms may not have passed through a phase comparable to the R form. In any case a new procedure for the transformation of pneumococcal types is presented.

5771

The Influence of Liver Extract and Acute Infection on Reticulocytes and Bone Marrow of Pigeons.

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The exact mechanism of the action of the potent material effective in pernicious anemia is still obscure. Certain facts are firmly established, however, such as the prompt reticulocyte response which this material induces, and that infection can inhibit this reaction to varying degrees. The potent material does not influence the peripheral blood of normal rats, rabbits, and dogs, nor, as a rule, does it effect anemias experimentally produced in these animals. However, pigeons seem to be peculiarly sensitive to the potent material. Relatively pure liver extracts injected intravenously, as well as commercial liver extracts fed by mouth, will cause a typical reticulocyte response in normal pigeons.¹ It has been tentatively suggested that this reaction is due to the partially megaloblastic type of bone marrow found in these birds.

To elucidate further the action of liver extract on the red blood cells in the bone marrow, and to obtain some possible explanation for the failure of an adequate reticulocyte response in pernicious anemia patients suffering from infections, the following experiments

¹Vaughan, J. M., Muller, G. L., and Zetzel, L., *Brit. J. Exp. Path.*, 1930, **11**, 456.

have been carried out: Three groups of pigeons, kept under standard laboratory conditions, were given, (1) a *staphylococcus aureus* infection intramuscularly, (2) liver extract No. 343, (N.N.R.) by mouth, and (3) both infection and liver extract. Reticulocytes were followed in the peripheral blood, and, at various stages, tissues from the hematopoietic system were studied for any histological changes. The radial bone marrow was compared throughout, as this, as a rule, is mainly fatty, and extension and hyperactivity when present, is therefore more marked.

The *Staphylococcus aureus* was obtained from a fatal case of human septicemia. After infection, there was, in many animals, a drop in the reticulocytes from normal, about 10%, to about 5% or lower, the lowest level being obtained from the 3rd to the 5th day. There followed a gradual rise of reticulocytes to a maximum of 25 to 30% between the 12th and 17th day after infection. Twenty-four to 48 hours after infection, active mitosis was observed in the primitive red blood cells lining the sinuses of the bone marrow, as well as in the leucocyte series of cells; and in about 5 to 7 days the red blood cell centers had enlarged enormously, many of them consisting of conglomerations of megaloblasts, with numerous mitotic figures. At a later stage, with the increased number of reticulocytes in the peripheral blood, the white blood cell hyperplasia had subsided considerably, while the red blood cell centers were large and contained cells in various stages of maturity.

Normal pigeons fed liver extract showed the characteristic reticulocyte response.¹ The bone marrow obtained after feeding liver extract daily for 4, 7, 11, and 15 days showed no extension of the hematopoietic tissue. The megaloblasts, however, were diminished in number. There was a decrease of hematopoietic activity and of number of mitotic figures. The red blood cell centers were smaller than normal, and most of the cells were in the late erythroblastic and normoblastic stage.

In the third group of birds the infection was superimposed after 3 to 7 days' administration of liver extract. The results in this group were, as was to be expected, somewhat mixed; but both in the peripheral blood and in the bone marrow, the reaction to the infection, as described above, seemed to predominate.

It therefore seems as if the physiological results on the hematopoietic organs from infection is the opposite of the effect of liver extract. Thus, acute infection in pigeons can produce a bone marrow analogous to the one seen in pernicious anemia, *i. e.*, with red blood cell hyperplasia, while liver extract given pigeons produces a

picture very similar to the one observed in patients with pernicious anemia after the administration of liver (Peabody²). Whether chronic infection acts in the same manner as acute infection is being investigated.

5772

Reticulocyte Response to Glutamic Acid in Pernicious Anemia.

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New York.*

The clinical trial of glutamic acid in cases of pernicious anemia as an antianemic substance was suggested by the following experimental observations. According to Abderhalden¹ glutamic acid may be condensed into pyrrolidonecarboxylic acid. Hemin, the iron porphyrin, synthesized by Hans Fischer² is composed of pyrrole rings. West, Howe and Dakin³ have recently isolated substances from liver tissue which are exceedingly active in pernicious anemia. These substances, identified by them as B-hydroxy-glutamic acid, δ -hydroxyproline, and as a tri-basic acid containing the pyrrolidone ring, are potential pyrrole precursors. More recently, Drabkin^{4, 5} and Miller⁴ found glutamic acid and certain other amino acids and compounds effective in the treatment of the anemia of rats caused by an exclusive milk diet.

Those substances which produce remissions in the blood picture of pernicious anemia, known to contain potential pyrrole precursors, are probably liberated from certain protein foods by normal gastric digestion⁶ and then decomposed so that their products are available after absorption for the synthesis of hemoglobin and erythrocyte stroma. In pernicious anemia the necessary substances and their de-

² Peabody, F. W., *Am. J. Path.*, 1927, **3**, 179.

¹ Abderhalden, E., and Kautzsch, K., *Z. f. physiol. Chem.*, 1910, **68**, 487.

² Fischer, H., and Zeile, *Ann. d. Chem.*, 1929, **468**, 98.

³ West, R., and Howe, M., *J. Biol. Chem.*, 1930, **88**, 427; Dakin, H. D., West, R., and Howe, M., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 2; Dakin, H. D., and West, R., *J. Biol. Chem.*, 1931, **92**, 117; West, R., and Howe, M., *J. Am. Med. Assn.*, 1931, **97**, 685.

⁴ Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531; **92**, 61 sup.

⁵ Drabkin, D. L., Discussion, *J. Am. Med. Assn.*, 1931, **97**, 686.

⁶ Castle, W. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sc.*, 1930, **180**, 305.

composition products are not made available to the organism in adequate amount due to some deficiency in the gastro-intestinal tract.

Ajinomoto (Suzuki) used extensively in Japan as a condiment for giving a meat-like flavor to foods is an exceedingly economical form in which to administer sodium-glutamate. If sodium-glutamate were to prove effective in the treatment of pernicious anemia, Ajinomoto would be a relatively cheap therapeutic agent. Forty grams of Ajinomoto was therefore administered in boiling water

TABLE I.
Hematological Observations Following Sodium Glutamate Feeding in Pernicious Anemia. Comparison with Liver Extract and Hog Stomach.

Case No.	Day of Treatment	Ajinomoto (Suzuki) 40 gm. daily			Day of Treatment	Liver Extract (Wilson)* 3-5 vials daily		
		HGB. %	R.B.C. Millions	Reticulo- cytes %		HGB. %	R.B.C. Millions	Reticulo- cytes %
1	Before	41	1.7	<1.0	Before	38	1.3	2.0
	2	—	—	2.0	2	38	1.6	1.0
	5	42	1.6	4.0	5	35	1.7	3.0
	6	—	—	5.0	7	40	1.9	5.0
	7	—	—	6.0	8	—	—	9.0
	9	38	1.3	6.0	10	43	1.9	3.0
	11	—	—	2.0	13	45	2.1	3.0
					15	45	2.0	<1.0
2	Before	39	1.7	1.0	Before	56	1.9	1.5
	1	45	1.6	2.0	2	64	2.0	4.0
	3	54	1.7	5.0	5	66	2.5	17.0
	5	56	2.2	4.0	8	70	2.5	—
	7	51	1.8	2.0	11	68	2.5	5.0
	9	54	1.8	1.0	14	70	2.6	5.5
	11	58	1.9	1.0				
	13	55	2.0	1.0				
	16	60	1.9	<1.0				
	19	60	1.9	2.0				
	25	58	1.9	1.5				
	27	56	—	—				
3	Before	27	0.9	<0.5	Before	42	1.6	2.0
	1	31	—	—	3	44	1.6	3.0
	3	—	—	1.0	7	44	1.9	3.0
	4	32	0.9	—	10	46	2.1	3.0
	5	30	1.0	—	15	52	2.8	5.0
	6	30	1.2	1.0	19	54	3.0	5.0
	9	30	1.4	—	22	53	2.9	2.0
	10	—	—	3.5	25	63	3.0	2.0
	11	34	1.6	—				
	13	—	—	3.0				
	14	40	1.7	2.0				
	17	42	1.6	2.0				
	Before	32	1.4	0.5	Before	32	1.1	2.0
4	2	35	1.3	3.0	1	29	1.7	1.0
	3	30	1.3	3.5	4	30	1.6	3.0
	5	28	1.1	2.0	6	38	1.8	2.4
	7	32	—	—	8	50	2.3	3.0
					21	60	2.7	2.4

* Hog stomach, 3 vials daily was employed in case 4.

in 4 equally divided doses daily to each of 4 patients suffering from pernicious anemia. In these doses the substance was well tolerated and in all instances promptly improved the appetite.

The hematological observations are recorded in Table I. The initial hemoglobin estimations made by the Sahli method on the 4 patients varied between 26 to 41%, the erythrocytes between 0.9 to 1.7 millions. After the treatment was instituted the maximum reticulocyte responses were 6.0, 5.0, 3.5, and 3.5% on the 7th, 3rd, 10th, and 3rd days respectively. The reticulocyte counts were made by Dr. Nathan Rosenthal. There were no rises in hemoglobin or erythrocyte counts except in case 2 where the hemoglobin mounted from 39 to 60% in 20 days without a corresponding increase in red blood cells. Ajinomoto ash contained only negligible traces of iron. Its copper content was not determined. The treatment with Ajinomoto was continued for 11 days, 27 days, 17 days, and 7 days respectively. Liver extract and hog stomach extract therapy was then instituted (Table I) with subsequent prompt rises in hemoglobin and erythrocytes.

Sodium-glutamate in the form of Ajinomoto is not an effective substitute for liver extract in the treatment of pernicious anemia. However, it is not completely devoid of activity, as indicated by minimal but definite reticulocyte response in 4 cases of the disease.

5773

A Recently Described Virus Disease of Parrots and Parrakeets Differing from Psittacosis.

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The widespread outbreak of psittacosis in 1929 and 1930 incited experimental work that quickly resulted in the discovery of the fact that the disease is caused by an agent capable of traversing bacteria-tight filters. Thus the first filterable virus indigenous to parrots was found. Since Amazon parrots were believed to be the chief source of the infection, workers in Brazil sought for evidence of psittacosis in their country. Pacheco, Bier, and Meyer¹⁻⁴ encoun-

¹ Pacheco, G., and Bier, O., *Compt. Rend. Soc. Biol.*, 1930, **105**, 109.

² Pacheco, G., *Compt. Rend. Soc. Biol.*, 1931, **106**, 372.

³ Pacheco, G., and Bier, O., *Arch. Inst. Biol., São Paulo*, 1931, **4**, 89.

⁴ Meyer, J. R., *Arch. Inst. Biol., São Paulo*, 1931, **4**, 25.

tered a disease in parrots that subsequently was shown to be induced by a filterable agent. According to their reports, the etiological agent passes Berkefeld N candles, is not cultivable on ordinary media, produces areas of necrosis in the liver and spleen in which are found acidophilic nuclear inclusions similar to those seen in *herpes febrilis* and Virus III infections, and is not pathogenic for pigeons, chickens, mice, guinea pigs, or monkeys. Moreover, these workers state that the clinical picture observed in infected birds is similar to that seen in avian psittacosis, and believe that the strict adaptation of the virus to the *psittacidae* accounts for the absence of human psittacosis in Brazil.

Dr. Pacheco sent me some of his virus in order that its activities might be compared with those of a virus known to have caused psittacosis in human beings. This comparison has been made, and I can confirm the results of the experimental work reported by the Brazilian investigators. The virus is not retained by Berkefeld V and N candles, nor is it cultivable on ordinary media. Parrakeets are susceptible, while canaries, chickens, rats, mice, guinea pigs, and rabbits are insusceptible. Numerous acidophilic nuclear inclusions are found in the specific lesions. I do not agree, however, with the conclusions that the strict adaptation of the virus to parrots and parrakeets accounts for the absence of human psittacosis in Brazil. On the contrary, my experiments with the virus indicate that another filterable virus disease of parrots has been discovered. Its difference from psittacosis is manifested by the facts that the causal agent is nonpathogenic for mammals and produces lesions in avian livers and spleens in which numerous acidophilic nuclear inclusions are found, instead of the "minute bodies" first described by Levinthal⁶ as characteristic of psittacosis.

5774

Cumulative Effects on Protein Utilization of Highly Milled and Whole Wheat Flours.

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When digestibility of food constituents is determined by the conventional method the experiment is of such short duration that

⁶ Levinthal, W., *Klin. Woch.*, 1930, **8**, 654.

only acute effects of the food on the animal have time to appear. Chronic effects are absent. Tests already reported¹ show that the habitual use by white rats of a ration consisting of white flour, potatoes, lean beef, milk, fats, and salts in the proportions customary for human dietaries, resulted, after several months, in a protein utilization somewhat lower than that found for a similar ration but containing whole wheat meal instead of white flour. Since white flour has been found to be more digestible than wheat meal² it seemed probable that a change gradually develops in the condition of the animal as a result of the continued use of the dietaries whereby digestion becomes less effective on the white flour ration or more effective on the wheat meal ration. To test these ideas digestion trials were made, at intervals, upon the same animals, maintained upon rations which varied only in the kind of flour used or in the amount of fiber or of yeast.

Five rations were compared. Ration I contained, white flour 45.5, dried potatoes 13.7, dried beef 11.9, sucrose 11.2, butter 5.2, lard 5.1, dried skim milk 4.7, and salt mixture 2.7. The crude fiber content of this ration is about 0.8%, protein 16.5%, and fat 12.0%. Presumably it is deficient in the antineuritic vitamin. Ration II contained enough ground filter paper to raise its fiber content to about 3.0%, otherwise it was like Ration I. Ration III contained both filter paper and 4.4% of dried brewers' yeast. Ration IV contained brewers' yeast but no paper. Ration V contained wheat meal in place of the white flour of Ration I. All 5 rations were fed to young white rats taken from the same litters, 3 rats being used on each ration. The digestibility of protein in the several rations at different times in the life of the rats is shown in Table I.

TABLE I.
Cumulative Effects of Wheat Flour, Cellulose or Yeast Upon Protein Digestibility.

Ration	Coefficients of Protein Digestibility After		
	1 month	4 months	6 months
White Flour	86.1	84.4	83.2
" " + Paper	83.5	82.7	81.6
" " " + Yeast	83.3	diarrhea	86.7
" " + Yeast	85.6	84.3	80.5
Whole Wheat Meal	77.1	88.0	86.9

As shown in the table, protein utilization decreased slightly as a result of the habitual use of white flour in the particular food com-

¹ Lyman, J. F., *Am. Med.*, 1928, **28**, 794.

² Atwater, Helen W., *Farmers Bul.*, 389, U. S. D. A.

binations used here. On the other hand the habitual use of whole wheat meal, in a similar food combination resulted in a very decided improvement in protein utilization. Whereas at the beginning of the experiment the protein of the white flour ration was much better utilized than that of the wheat meal ration, at the end of 6 months the digestibility coefficient of the latter exceeded the former. The effect of additions of cellulose only and of yeast only upon protein utilization was detrimental and this effect was cumulative. Cellulose and yeast together, added to the white flour ration, appears to be somewhat beneficial upon protein utilization, although the condition of the animals fed this combination was not as good as that of the other groups and there was some diarrhea, something never observed with the other diets.

5775

Avitaminosis. VI. Vitamin B Lipemia Versus Inanition Lipemia in the Lactating Rat and its Nursing Young.*

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The purpose of this study was to determine the effect of a deficiency of vitamin B *per se* on lipid metabolism of the lactating albino rat and its nursing young. The avitaminosis was produced according to technique previously described.¹ The experiments were conducted in triads. The pathological group received autoclaved dried baker's yeast as a source of vitamin G. Litter mates were restricted to the same amount of food and water of the same diet as consumed by the avitaminotic group, but the yeast in the ration was untreated. The plane of nutrition was the same in this diet, and the only limiting factor was the destruction of vitamin B in the process of autoclaving. Another group of litter mate lactating rats was fed the same ration, containing the untreated yeast, but the daily intake of food and water was unrestricted. As the only limiting factor between the second and third groups was the plane of nutrition, the difference is due to inanition.

One-half a cc. of blood was used for the determination of fatty

* Research Paper No. 238, Journal series, University of Arkansas.

¹ Sure, B., and Smith, M. E., *J. Nutr.*, 1929, **1**, 537.

acids, lecithins, and iodine number. When the cholesterol was determined it was obtained from a separate one-half cc. sample, because results were not satisfactory when cholesterol was first obtained by chloroform extraction and the residue used for the determination of the other lipid constituents.

Nine triads, each consisting of pathological, restricted, and unrestricted mothers with their litters, were studied. An analysis of the data obtained from these groups made the following conclusions possible: Vitamin B deficiency *per se* produces a very pronounced influence on the concentration of fatty acids in the blood of the lactating albino rat and its nursing young. It is also evident from determinations of the iodine number that a great proportion of the fatty acid increase belongs to the unsaturated series. The lecithins show a rise but to a much lesser extent. There is a definite rise in cholesterol of both mother and young as a result of vitamin B deficiency, but more data must be obtained before final conclusions can be drawn.

The effect of the deficiency of vitamin B *per se* in the diet of lactating mothers and nursing young can be more readily visualized by an examination of Table I, which shows the increase per cent of the lipid constituents of the blood of the pathological groups over the restricted groups.

TABLE I.

	Fatty Acids	Iodine No. of Fatty Acids	Cholesterol	Lecithins
	%	%	%	%
Mother	29	27	25	10
Nursing Young (Litters)	48	37	32	16

Inanition also produces an increase per cent of the lipids of the blood of restricted groups over the unrestricted mothers and litters, but to a much lesser extent than vitamin B *per se*. This is shown in Table II.

TABLE II.

	Fatty Acids	Iodine No. of Fatty Acids	Cholesterol	Lecithins
	%	%	%	%
Mother	18	18	6	7
Nursing Young (Litters)	19	12	0	9

It is quite apparent from the data that the lipemia found in vitamin B deficiency is not due to greater concentration of the blood,

as the blood of the restricted groups was either of the same or slightly higher concentration than the pathological groups.

All the lipemia could not be attributed to catabolism of body tissue because some of the mothers on a vitamin B deficient ration, owing to previous storage, did not decrease in body weight as much as their corresponding restricted controls.

For the past 4 years attempts were made to find a symptom complex in vitamin B deficiency produced in albino rats, as evidenced by the blood picture. The anhydremia and increase of non-sugar reducing substances in the blood were considered of interest but of relatively little clinical significance. The findings of a marked lipemia in this avitaminosis, reported in this communication, may prove of aid to the diagnostician in vitamin therapy.

5776

Glycogen Formation after Oral Administration of Sodium Salts of Propionic, Butyric, Valeric and Caproic Acids.

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By the use of Cori's method, the absorption of and the formation of glycogen from the salts of fatty acids of low molecular weight have been determined. Male rats previously fasted for 24 hours were used. The control and experimental animals although not litter mates were nearly of the same age and weight. The data in the table show that of the substances administered only sodium propionate affected the glycogen stores. The increments in liver glycogen resulting therefrom, although not large, are nevertheless definite, and are not to be ascribed to individual variations. The failure of butyric and caproic acids to form glycogen cannot be due to faulty absorption, since it was demonstrated that their salts were as well absorbed as sodium propionate. The data are sufficient to warrant the inclusion of propionic acid in that list of products that can be transformed into glycogen by the white rat. Butyric and caproic acids cannot be added to that list for under the conditions obtaining in this study no evidence of the formation of glycogen from these acids was secured. Further studies on the behavior of valeric acid are desirable, since it is possible that the dosage em-

ployed was too small. Until such studies are completed definite conclusions regarding the fate of this acid must be withheld.

TABLE I.

	No. of Animals	Liver Average %	Glycogen, entire body except liver Average %
Fasting controls	32	0.070 (0.036-0.093) *	0.067 (0.049-0.092)
Sodium propionate	21	0.313 (0.092-1.370)	0.077 (0.061-0.104)
" butyrate	5	0.063 (0.057-0.069)	0.054 (0.043-0.065)
" valerate	6	0.096 (0.075-0.122)	0.076 (0.064-0.091)
" caproate	8	0.084 (0.046-0.098)	0.074 (0.059-0.071)

* These are the ranges in the individual experiments.

5777

Results of Feeding Staphylococcus Filtrates to Monkeys.

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Attempts to produce in the lower animals symptoms resembling those in human volunteers fed with staphylococcus filtrates have not generally been successful. Barber stated that kittens, puppies and monkeys show no symptoms after ingesting large quantities of milk cultures of the toxic staphylococcus isolated by him: "or at all events, the symptoms are very slight." Unpublished work by Dack and others in the Bacteriology Laboratory of the University of Chicago has similarly given negative results. Rhesus monkeys of approximately 6 kilos have repeatedly been fed large amounts—up to 60-70 cc.—of staphylococcus filtrates of proved toxic power for man without in any instance producing the "food poisoning" symptoms observed in human volunteers. These consistently negative results led us to abandon for a time attempts to produce illness in monkeys by feeding toxic filtrates.

In March, 1931, opportunity was offered us through the courtesy of Dr. H. C. Clark, Director of Gorgas Memorial Laboratory, Panama, of repeating these experiments with a number of smaller monkeys of different species. The monkeys used were chiefly the red spi-

der (*Ateles geoffroyi*), black spider (*Ateles ater*), black howler (*Alouota palliata inconsonans*), and white face (*Cebus capucinus capucinus*). They were nearly all juvenile, weighing between one and 2 kilos. The filtrates fed were from staphylococcus broth cultures after 2 to 3 days' growth; their gastro-intestinal toxicity for man was in each instance established by their effect in 2 cc.-10 cc. amounts on human volunteers. Thirteen monkeys were fed amounts ranging from 5 cc.-20 cc. A rubber catheter was introduced into the stomach, and measured amounts of the filtrate were slowly injected by a graduated syringe. In five (2 red spiders, 2 black howlers, 1 black spider) definite symptoms were observed of a similar character to those described in human subjects.¹ These animals suffered from marked diarrhea and showed lack of appetite; they remained huddled in a corner and apparently experienced abdominal pain. The onset of the symptoms occurred about 2 hours after the filtrate was administered, corresponding to what is observed in man. In one instance vomitus was found on the floor of the cage. The condition of malaise lasted for approximately one day, after which recovery appeared to be complete. In one animal, however, the diarrhea persisted for about 8 days. In 3 other animals symptoms of slight malaise were noticed; these were perfectly definite in character, such as weakness, sluggishness and lack of interest in food, but were not accompanied by active gastro-intestinal disturbances. The other 5 animals were not visibly affected by the feeding. One of the monkeys that had diarrhea after being fed 10 cc. of filtrate on March 2 manifested slight malaise when given 20 cc. of a less potent filtrate 10 days later.

5778

Changes in the Reducing Substances in the Liver and Muscle in
Experimental Streptococcus Septicaemia.

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This paper deals with the effect of experimental streptococcus septicaemia upon reducing substances in the liver and muscle of rabbits. It was recently shown¹ that during the course of such an

¹ Jordan, Edwin O., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 421.

¹ Linton, R. W., *J. Exp. Med.*, in press.

infection the blood sugar tends to become less concentrated. The average blood sugar level for normal rabbits was 135 mg. per 100 cc. of blood, using the micromethod of Folin.² At the terminus of the experimentally induced septicaemia, which always ends fatally, the average blood sugar was about 30 mg. per 100 cc. less than the normal.

While the terminal concentrations of blood sugar are not at hypoglycaemic levels, it was nevertheless thought of interest to study the decrease further, especially in view of the fact that the other blood changes pointed to a large production of acid *in vivo* by the streptococcus. Since the work of Hopkins and Parker,³ it has been recognized that the tissues are the site of infection in experimental streptococcus septicaemia. The defenses of the body are in the tissues, and here also the organisms proliferate, those appearing in the blood having been passively carried away from tissue foci of infection. In spite of the profound changes which occur in the blood,⁴ it is felt that these alterations simply reflect the more fundamental disturbances occurring in the tissues.

Septicaemia was produced in rabbits by the intravenous injection of from 0.5 cc. to 0.75 cc. of a 24-hour broth culture of *Streptococcus hemolyticus*. In the 7 experimental animals the resulting infections reached fatal terminations in from 2 to 8 days. Tissues were removed from the rabbits just at death, or the animals were killed at a period when the non-protein nitrogen had risen to an abnormal level. It was found in the earlier work that a rise in this constituent could be used as a guide to the time at which the animal had entered the terminal period of the disease, and had an expected survival of only a few hours. The 8 animals in the control series were killed by a blow on the neck. They had not been starved before their tissues were taken. Ten to 12 gm. of liver, and 6 to 8 gm. of muscle from the abdominal wall were taken from each rabbit. In the experimental animals, heart's blood cultures were positive for *Streptococcus hemolyticus* in each case.

The extraction of the tissues, precipitation of the protein, and determination of the total reducing substance, non-fermentable substance, and true sugar were carried out according to the procedure of Trimble and Carey.⁴

The amounts of total reducing substance, non-fermentable substance, and true glucose in liver and muscle are given in Tables I and

² Folin, O., *J. Biol. Chem.*, 1928, **77**, 421.

³ Hopkins, J. G., and Parker, J. T., *J. Exp. Med.*, 1918, **27**, 1.

⁴ Trimble, H. C., and Carey, B. W., Jr., *J. Biol. Chem.*, 1931, **90**, 655.

II. The former contains the figures for the normal series, and the latter the data on the infected animals. Table II also shows the length of survival after the injection of the streptococcus.

TABLE I.
Total Reducing Substance, Non-fermentable Substance, and True Sugar in the Liver and Muscle of Normal Rabbits, in mg. per 100 gm. of Fresh Tissue.

Rabbit No.	Liver			Muscle		
	Total Reducing Substance	Non-fermentable Substance	True Sugar	Total Reducing Substance	Non-fermentable Substance	True Sugar
1	64.52	7.55	56.97	8.61	5.69	2.91
2	58.3	6.4	51.9	9.27	4.67	4.59
3	47.4	6.6	40.8	10.7	6.2	4.5
4	95.2	9.95	86.25	8.75	4.45	3.65
5	76.8	7.9	68.9	7.0	—	—
6	45.0	4.2	40.8	9.0	—	—
7	56.18	6.49	49.69	6.79	3.89	2.9
8	43.19	4.78	38.41	7.28	4.16	3.12
Average	60.82±3.91	6.73±0.38	54.21±3.91	8.42±0.33	4.84±0.22	3.61±0.18

TABLE II.
Total Reducing Substance, Non-fermentable Substance, and True Sugar in the Liver and Muscle of Rabbits in the Terminal Stage of Septicaemia due to *Streptococcus hemolyticus*, in mg. per 100 gm. of Fresh Tissue.

Rabbit No.	Liver			Muscle			Survival after Infection. Days
	Total Reducing Substance	Non-fermentable Substance	True Sugar	Total Reducing Substance	Non-fermentable Substance	True Sugar	
1	35.64	3.49	32.15	5.14	3.31	1.83	8
2	23.31	5.24	18.07	4.14	3.88	0.26	5
3	14.05	3.51	10.54	2.57	1.41	1.16	2
4	36.1	5.89	30.21	4.1	3.3	0.8	3
5	33.1	5.15	27.95	—	—	—	3
6	17.75	5.08	12.67	3.56	2.29	1.27	3
7	9.48	3.16	6.32	3.57	3.18	0.39	3
Average	24.2±2.56	4.5±0.24	19.7±2.4	3.84±0.2	2.89±0.21	0.95±0.14	

The relations between the total reducing substance, non-fermentable substance, and true sugar in the livers and muscles of the 2 groups may be expressed as follows: The non-fermentable substance of normal rabbit liver is approximately one-tenth of the total; in infected rabbits it is more variable, but usually represents one-fifth or one-sixth of the whole. That is to say, while both constituents are reduced, the true sugar undergoes a greater proportionate reduction than the non-fermentable substance. The actual reduction can be estimated by a comparison of the average values. The average for the total reducing substance drops from 60.82 mg. to 24.2 mg.,

for the non-fermentable substance from 6.73 mg. to 4.5 mg., and for the true sugar from 54.21 mg. to 19.7 mg.

The data derived from the study of the muscles lead to similar deductions. In the normal animals, the non-fermentable substance makes up slightly more than 50% of the total; infected animals' muscles, on the other hand, have about three-quarters of the total reducing substance in the form of non-fermentable constituents. Here again the proportionate reduction is greater for the true sugar than for the non-fermentable substance. The actual reduction, in so far as it is shown by the averages, goes from 8.42 mg. to 3.84 mg. for the total reducing substance, from 4.84 mg. to 2.89 mg. for the non-fermentable substance, and from 3.61 mg. to 0.95 mg. for the true sugar.

In the work reported above,¹ it was suggested that the production of organic acids *in vivo* by the streptococcus played an important rôle in bringing about the death of experimentally infected animals. In view of the fact that the organisms grow in the tissues, especially in the muscles, in contrast to the blood, in which they probably do not grow, the present findings suggest strongly that tissue glucose serves as the source of the acids produced by the streptococcus. Under these conditions, the gradually lowered blood sugar would result from an increasingly large transfer of glucose from blood to tissue, with a consequent drain on the liver. In this connection it is interesting to note that the protein-free extracts of infected liver are water-clear, while similar extracts of normal liver have the decided opalescence of glycogen solutions. Hopkins and Parker³ have shown that the liver of even a susceptible animal will destroy large numbers of streptococci in the early part of the infection, while the organisms begin at once to grow in the muscles. Thus the liver, since it contains few parasites or none, and is therefore not being drained of glucose by their growth, is in a position to furnish sugar to the muscles where proliferation is rapid and destruction of the organisms slight or absent. In the last stage of the disease, however, the streptococci begin to proliferate in the liver itself, and the glucose concentration of the blood is still further lowered. The evidence is not clear for a separate "toxic effect" of the streptococci, which might be supposed to act apart from and in addition to the purely biochemical changes in bringing about the death of the animal.

Conclusion. The concentrations of total reducing substance, non-fermentable substance, and true glucose have been determined in the livers and muscles of rabbits in the terminal stages of acute strep-

tococcus septicaemia. These concentrations are found to be significantly lower than those in normal rabbits.

5779

A Thermoregulated Foot Cradle for the Treatment of Peripheral Vascular Disease.

ISAAC STARR, JR. (Introduced by H. C. Bazett.)

From the Medical Division of the Hospital of the University of Pennsylvania.

When the circulation to an extremity is impeded the ability to maintain the proper temperature of the part may be seriously impaired. Therefore it seemed that patients with gangrene of the feet due to peripheral vascular disease might be benefitted by discovering and maintaining the temperature optimum for the circulation in their feet. This preliminary communication is submitted because of the relief from pain which has followed this procedure in 3 cases, two of diabetic and one of arteriosclerotic gangrene.

Each patient had a spot of dry gangrene on one great toe. The local lesions had been treated by placing the feet in a bed cradle containing an electric bulb permanently lighted, but pain had persisted.

Procedure. The threatened feet were placed in a large water bath equipped with a stirring device and heating unit. At the initial temperature, about 31° , the patients complained that the cold caused pain, and the skin was pale and blue with patches of deeper cyanosis near the gangrenous areas. As the temperature was gradually raised (about 1°C. in 5 min.) the pain lessened and the color improved. At a temperature of 34° in 2 patients, 35° in the third, pain disappeared completely and the color of the feet approached the normal. After the temperature had risen another degree pain and cyanosis returned. Increasing pain and deepening cyanosis accompanied a further rise of temperature. Therefore 34° or 35° appeared to be the optimum temperature for the feet of these patients.

After discovering the optimum temperature, a foot cradle* equipped with a thermoregulator was placed upon the patient's bed and the gangrenous foot kept in it constantly. The thermometer

* This cradle was demonstrated at the scientific exhibit of the American Medical Association meeting at Philadelphia, June, 1931.

was set to maintain the air at $1^{\circ}\text{C}.$ above the temperature optimum for that foot in water (to allow for the cooling effect of evaporation from the skin). In 2 cases relief of pain promptly followed. The third patient was much more comfortable but attacks of pain persisted; therefore the air temperature was changed from 35° to 37° , then back to 35° , and finally to 33.5° . This last temperature was accompanied by almost complete relief. In all these cases the gangrene cleared up without amputation.

Conclusions. The optimum temperature for the feet of my patients proved to be considerably below their mouth temperature. It corresponded closely with the skin temperature of the feet of normal persons. When this temperature was rigidly maintained comfort was at a maximum and the color of the feet most closely approached the normal. Slight deviations from it were accompanied by pain and cyanosis.

It appears reasonable to believe that strict maintenance of the optimum temperature would increase the chances of recovery in many cases of gangrene secondary to peripheral vascular disease.

5780

Closed Loop Fluid.*

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In the course of work previously reported¹ on closed loops of small intestine, observations were made upon the nature of the fluid aspirated from the distended loop after complete recovery of the animal. While a closed loop may not show any accumulated fluid for weeks at a time it may at intervals become filled with fluid, which must be aspirated to prevent distension and necrosis of the loop. This fluid is gray in color and has a specific gravity of 1.012 (average of 108 specimens).

The reaction, determined by the quinhydrone electrode, varied within relatively wide limits: 47 jejunal loop specimens gave an average pH of 7.5 with extremes of 9.0 and 6.3; 51 ileal loop specimens gave an average of 7.7 with extremes of 9.0 and 6.2. All

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

¹ Burget, Martzloff, Thornton and Suckow, *Arch. Int. Med.*, 1931, **47**, 593.

determinations were made immediately after withdrawing the fluid.

In testing for enzymes the following substrates were used: 1% sucrose solution, 5% peptone and olive oil. The sucrose activity was estimated by determining the amount of reducing sugars by the Schaffer-Hartman method. Peptone hydrolysis was estimated by the formol titration and lipase activity measured by titrating the fatty acids liberated with alcoholic KOH. Seventy-four ileal specimens were tested and 104 jejunal specimens. Sucrase and erepsin activity were demonstrated in 50% of the ileal specimens and in 75% of the jejunal specimens. Lipase was only occasionally present.

These results show that the fluid accumulated in a distended loop is not pure *Succus entericus*. Some factor has brought about abnormal secretion together with some filtration into the loop. Normal loops absorb all fluid leaving a slowly accumulating pastelike debris.

5781

Neutralization of Virus of Poliomyelitis with Serum of Healthy Porto Ricans.

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The geographic and seasonal incidence and variations of poliomyelitis have been among the most interesting problems in the study of the epidemiology of this disease. Further, the preponderance of cases in children under the age of puberty with also a tendency towards a larger proportion of cases in males than in females is striking. Friedberger, Bock and Furstenheim¹ have called attention to antibody rise in relation to age and one may logically inquire what, if any, may be the physiological factors involved which may modify the susceptibility or resistance to infection with the virus of poliomyelitis. The work of Aycock² and of Shaughnessy, Har-

¹ Friedberger, E., Bock, G., and Furstenheim, A., *Z. f. Immun. Forschung*, 1929, **64**, 294.

² Aycock, W. Lloyd, *J. Prev. Med.*, 1929, **3**, 245; *Am. J. Hyg.*, 1928, **8**, 35; and Kramer, S. D., *J. Exp. Med.*, 1930, **4**, 457.

mon and Gordon³ indicates that the serums of normal individuals, particularly adults, may in a high percentage of cases neutralize this virus. Aycock has suggested that different reactions to infection with poliomyelitis virus may be due to physiological variations in the host, rather than to variations in the virus itself. Further this author states that evidence that immunity to poliomyelitis is as common in warm climates as in cool climates led to the belief that the virus is probably as widely distributed in warm regions as in cool, and also that the lower incidence of the disease in warm regions is probably not due to a corresponding diminution of the virus in such regions. The work of the authors cited suggests that neutralizing substance for poliomyelitis virus found in the serums of normal people is of a specific nature and therefore due to exposure to the virus. This seems, of course, to be the most logical assumption.

The physiological effects of warm climates on man still constitute a subject for debate among scientists who have studied this problem. That such effects do exist seems now to be established but to what factors or combination of factors responsible for these effects we are still in ignorance. How these physiological modifications may influence man's response to infection, if at all, remains undetermined though we believe that certain diseases are definitely modified in tropical environments or, for some reason, do not exist at all. Scarlet fever, for instance, is a pertinent example.

Poliomyelitis is a disease chiefly of temperate climates and its incidence in warm climates, particularly the tropics, is exceedingly low. Yet cases of the disease, and even epidemics, have been reported in semitropical countries—but rarely so. Poliomyelitis is rare in Porto Rico. No clinical cases of this disease originating in the Island have been proved at autopsy. We have thought it of interest to test the serums of a group of healthy Porto Ricans for neutralizing substance for this virus. In all, 8 normal people have been studied. None of these individuals gave any history of contact with cases of paralysis nor did they have any history or symptoms of paralysis themselves. The serums from these cases were tested in dilutions as high as 1:30. Serums and virus were mixed in 0.5 cc. quantities and incubated for 2 hours at 37°C., placed in the ice box for 18 hours and then tested in *Macacus rhesus* monkeys by intracerebral injections of 1 cc. of the serum-virus mixture. Control animals received similar dilutions of both filtered and unfiltered virus following 2 hours' incubation and 18 hours in the ice

³ Shaughnessy, H. K., Harmond, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, 4, 463.

box. The control animals developed symptoms of poliomyelitis on the ninth day while the test animals remained entirely well for over 3 months during which time they were under daily observation. The serums employed from the healthy individuals therefore contained neutralizing substance for the virus of poliomyelitis. Are such neutralizing antibodies in the serums of such individuals specific? Are they due to acquired immunity through coming in contact with the virus of poliomyelitis? Or are such antibodies (if they are true antibodies) due to physiological factors as yet unknown? Should such phenomena be classified under the head of "physiological immunity"? It is true the experiment reported is based upon a small number of cases but they were chosen at random and they represent a 100% result. They are reported at this time simply to raise the question of specificity. The appearance of neutralizing substance for poliomyelitis virus in adult Porto Ricans may be of specific nature but we feel that certain physiological factors should also be studied in connection with this problem.

5782

Effect of Acid Extract of Anterior Pituitary on Iodine Content of Blood and Thyroid in Guinea Pigs.*

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Loeb, Bassett and Friedman, Siebert and Smith, and Martin Silberberg^{1, 2, 3, 4, 5} have shown that injections of acid extract of cattle anterior pituitary produce changes in the guinea pig which bear

* These investigations were in part carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

† We are indebted to Miss Hilda Friedman for her assistance in these experiments.

¹ Loeb, Leo, and Bassett, R. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 860; 1930, **27**, 490; Loeb, Leo, Bassett, R. B., and Friedman, Hilda, *Ibid.*, 1930, **28**, 209.

² Siebert, W. J., and Smith, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 622.

³ Siebert, W. J., and Smith, R. S., *Am. J. Physiol.*, 1930, **95**, 396.

⁴ Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 166.

⁵ Silberberg, Martin, *Krankheitsforsch.*, 1930, **8**, 171.

close resemblance to the changes found in man in cases of Graves disease, as far as the structure of the thyroid gland, the behavior of the colloid, the changes in weight, as well as in the basal metabolism and the effect of KI in this condition are concerned. Lunde, Closs and Wülfert⁶ have shown that the acetone insoluble or globulin-iodine fraction of the thyroid gland of toxic goiters is both absolutely and relatively decreased, while the total blood iodine concentration has been found by Lunde, Closs, Pedersen,^{7, 8} and others^{9, 10} to be increased. There is reason for assuming that an increased elimination of the thyroid hormone from the gland into the circulation takes place in Graves disease. We wished to determine whether similar changes in the iodine distribution occur in the guinea pig under the influence of acid extracts of cattle anterior pituitary.

We carried out 2 experiments. In the first, 8 guinea pigs, ranging in weight between 170 and 210 gm., were injected 5 times over a period of 6 days with 1 cc. of the acid extract. The pooled thyroid glands and 8 blood specimens of these animals were analyzed for their content in alcohol soluble and insoluble iodine. The thyroids and blood from 12 control guinea pigs of a similar weight were analyzed in the same way. In a second experiment, corresponding analyses of the blood and the thyroid glands from 12 injected and 12 control guinea pigs were carried out. In this case 6 samples of blood were analyzed separately from controls and 6 samples from the injected animals. In this second experiment the animals received one injection daily for 6 days. The iodine was determined according to v. Fellenberg's method¹¹ as modified by Lunde and Closs¹² and according to further modification by Closs.^{13, 14}

In the first experiment the alcohol insoluble fraction of iodine of the thyroid glands decreased 62% per unit of weight of the glands and, in the second experiment, it decreased 85% per unit of weight. Since as a result of injections of the extract the weight of the thyroid gland increases considerably in the guinea pigs, the decrease

⁶ Lunde, G., Closs, K., and Wülfert, K., *Biochem. Z.*, 1929, **206**, 248.

⁷ Lunde, G., Closs, K., and Pedersen, O., *Chr. Biochem. Z.*, 1929, **206**, 261.

⁸ Holst, J., Lunde, G., Closs, K., and Pedersen, O., *Chr. Klin. Wochenschr.*, 1928, **1**, 2287.

⁹ Veil, W. H., and Sturn, A., *Dtsch. Arch. Klin. Med.*, 1925, **147**, 166.

¹⁰ Kraft, R., *Mittell. Grenzgeb. inn. Med. Chirurg.*, 1927, **40**, 433.

¹¹ v. Fellenberg, *Th. Ergebnisse der Physiologie*, 1926, **25**, 176.

¹² Lunde, G., and Closs, K., *Norsk. Mag. Laegevid.*, 1928, **6**, 500.

¹³ Closs, K., *Arch. f. Math. O. Naturvid.*, 1931, **40**, 5.

¹⁴ Closs, K., *J. Pharm. and Exp. Therap.*, in press.

in the alcohol insoluble iodine per total gland was somewhat less, namely 23% and 69% respectively; nevertheless there was here a distinct loss as a result of the anterior pituitary administration. In the blood on the contrary, the alcohol insoluble iodine showed a rise of 82% in the first experiment and of 179% in the second experiment. In this case a few individual determinations which for technical reasons were unreliable were omitted. If they are included the increase in the first experiment was 34%. The determinations of the alcohol soluble iodine did not indicate a noticeable change either in the thyroid gland or in the blood.

These findings of a decrease in the alcohol insoluble fraction of iodine in the thyroid glands harmonize well with the histological changes which result from the injections of acid extracts. As Loeb and Bassett¹ have shown, the hypertrophy of the thyroid gland produced under these conditions is associated with a marked decrease in the amount of colloid in the acini and, what is left of it stains very poorly with eosin, a condition pointing to a marked liquefaction and absorption of the colloid. Inasmuch as the iodine in the thyroid is mainly contained in the colloid, the fact that by far the greater part of the colloid in the individual acini has been liquefied and absorbed justifies the assumption that a diminution in the amount of iodine has taken place in the thyroid, notwithstanding the marked proliferation of the acinar epithelium and the resulting increase in the amount of gland tissue which has taken place as a result of the administration of anterior pituitary extract.

These experiments which we are extending at the present time, strongly suggest the conclusion that extracts of cattle anterior pituitary produce in the organism of the guinea pig changes in the iodine distribution which correspond to those noted in man in cases of Graves disease.

5783

Long Continued Injections of Acid Extract of Anterior Pituitary on Thyroid Gland and Sex Organs.*

LEO LOEB AND HILDA FRIEDMAN.

From the Department of Pathology, Washington University School of Medicine.

In former experiments on the effects of the injection of acid extract of cattle anterior pituitary on the thyroid gland of guinea

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

pigs, we had found indications that these effects did not increase with continued injections. To determine the effects of long continued administration, a considerable number of young guinea pigs were given daily injections of anterior pituitary extract over a long period of time. Excluding those animals which were examined within the first 6 weeks after the beginning of the experiment, 10 guinea pigs received injections during a period of time varying between 47 and 74 days. The individual amounts injected daily varied between 1 cc. and 3 cc. of the extract. In some cases a few initial doses of $\frac{1}{2}$ cc. were given. The total amounts injected ranged between 47 and 135 cc. The initial weights of the individual guinea pigs were between 185 and 205 gm. except one animal weighing 230 gm. There was a great difference between the weights reached at the end of the experiment. Some animals had gained considerably, while others remained unchanged.

The microscopic examination of the organs showed that the severity of the effects of extract did not correspond to the amount injected. The animals evidently varied greatly in resistance. The resistant animals showed the least changes in their organs and these gained most rapidly in weight, while the most susceptible ones showed the greatest changes in their thyroid and sex organs, and did not gain as much or as rapidly in weight as the others. The former group became most readily adapted even to increasing doses of the extract. Thus those animals receiving the largest daily doses gained most in weight and their thyroid and sex organs showed the least change from normal or no change at all.

Effect on the Thyroid Gland. The relation between gain in weight and lack of hypertrophy in the thyroid can readily be seen. The thyroids of those animals which tolerated the largest doses of extract and gained most in weight showed least hypertrophy or none at all. The colloid was normal in quantity and of normal consistency. On the other hand, with one apparent exception, the glands of those that gained least in weight showed the greatest hypertrophy and their acini contained much less colloid. But even in those cases in which the thyroid showed definite hypertrophy and in which as a result of thyroid hypertrophy there was least gain in weight, there is apparent a relative increase in colloid due to new formation of this substance and some reduction in hypertrophy as compared to the changes in the thyroid gland of control guinea pigs which received only 6 to 8 injections of acid extract.

Effect on Sex Organs. We may distinguish 3 types of effects. (a) The sex organs were found normal. In the ovary the follicles

matured and ovulation took place, vagina, cervix and uterus behaving in accordance with the stage of the sexual cycle. These were the effects in guinea pigs in which there was considerable gain in weight, and in which the thyroid showed no hypertrophy. (b) In the animals which did not gain in weight or gained little, the thyroid gland (with one possible exception) showed hypertrophy. The ovaries were hypotypical, contained much interstitial gland and, in one case also a pseudolutein body. Vagina, uterus, and mammary gland were in a resting condition. (c) There was an intermediate group in which the guinea pigs gained much in weight, but less than in Group A. The thyroid showed no or only a trace of hypertrophy. The follicles developed well in the ovaries and proestrus as well as ovulation could occur, but at the same time interstitial gland and pseudolutein bodies or the latter alone formed. The other sex organs behaved normally, except that we found a quiescent vagina in a case in which both interstitial gland and pseudolutein tissue were present. Hypertrophy of the thyroid and the resulting deficiency in weight caused a hypotypical condition of the ovaries; in addition the acid extract was responsible more directly for the formation of interstitial gland and pseudolutein bodies.

We may conclude that after a long continued series of injections of acid extract of cattle anterior pituitary a gradual adaptation, partial or even complete, to the effects of the injections may take place in young guinea pigs and that there seems to exist a considerable difference in the ability of individual animals to acquire this increased tolerance. We may attribute the hypotypical condition of the ovaries largely to the relative undernourishment caused by the induced hypertrophy of the thyroid gland.

5784

Production of Experimental Polycythemia with Cobalt.

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Colorado School of Medicine, Denver.*

During a continuation of our studies on nutritional anemia, a polycythemia was observed in rats receiving a certain mixture of metals with Cu as a supplement to a milk-Fe diet, whereas the same

mixture of metals without Cu failed to produce this condition. The data are reported together with those from a study of the metals or combination of metals responsible for the polycythemia.

Male albino rats, 26 days old, were placed in glass cages and fed whole milk for 4 days. A mixture of Mn, Co, Ni, Zn, Cu, and Fe salts was then added as a supplement to the milk diet 6 days a week. All metals were fed as the same preparations, and at the lower levels, used in our previous work.¹

Weekly hemoglobin determinations showed normal values until the animals were 7 to 9 weeks of age, when a definite increase in hemoglobin occurred. The rats presented an extremely ruddy appearance, and their eyes, ears, and paws became deep red. When it was evident that this was not a transitory condition, hematological studies were made on the animals of this group, as well as on 2 control groups—one receiving the milk diet supplemented by Cu and Fe only, and the other, a normal stock diet. The group averages, based upon 3 determinations at weekly intervals on each rat, may be summarized as follows:

TABLE I.

Diet	No. of Rats in Group	Hemoglobin (gm. per 100 cc.)	Erythrocytes (per cu. mm.)	Cell Volume %
Stock	6	13.5	9,436,945	53.6
Milk-Fe-Cu	6	12.2	8,547,778	48.6
Milk-Fe-Cu + Mn, Co, Ni, Zn	5	19.4	14,599,000	76.5

The table shows that the animals on the entire mixture of metals exhibited a marked polycythemia, whereas the rats of either control group showed none. The animals showing polycythemia are now 275 days old and the condition has persisted for 30 to 32 weeks. Another group of rats, not reported here, received the mixture of metals without Cu and developed a progressive anemia rather than a polycythemia. Thus, it is apparent that one of the metals—Mn, Co, Ni, or Zn—or some combination of these metals, acts with Cu to produce a polycythemia in rats on a milk-Fe diet.

To determine which one or what combination of the 4 metals mentioned above was responsible for the polycythemia, 2 rats were placed on each of the 15 possible combinations of the 4 metals—Mn, Co, Ni, and Zn—as a supplement to a milk-Fe-Cu diet. Three animals on this basal diet and 7 on a stock diet were maintained as controls. In every one of the 16 animals that received *cobalt*, there

¹ Underhill, F. A., Orten, J. M., and Lewis, R. C., *J. Biol. Chem.*, 1931, **91**, 13.

appeared the same marked increase in hemoglobin, erythrocytes, and cell volume as was previously observed, whereas none of the other 24 animals showed any increase above normal values. The total and differential leucocyte counts in this and in the previous experiments showed similarity between the different groups of animals. Further work of a preliminary nature with CoSO_4 , used in place of the CoCl_2 , has shown that it is also effective in producing a polycythemia. Thus, it is definitely established that Co acting with Cu as a supplement to a milk-Fe diet is the causal agent in producing the polycythemia observed in our rats.

In a study of the toxicity of metals, Waltner and Waltner² have reported a polycythemia in the rat after feeding metallic cobalt and after the injection of Co salts in significantly larger doses than those given in our experiments.

5785

Grafting of Larval and Adult Eyes in *Amblystoma Punctatum*.

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Replanted and transplanted eyes of *Amblystoma punctatum* were studied daily for many months in 193 larval grafts on larval hosts around 21 mm. in length and in 102 adult grafts on adult hosts varying in age from 1 to 2 years and in length from 6 to 18 cm. Since no striking differences exist based merely on replantation and transplantation, a comparison is given based on the age of the graft. Return of circulation after operation was obtained in 40% of old adults, where sloughing or partial resorption was high, against 80% of young adults. Its earliest appearance was on the first day in young adults and on the third day in old adults. The earliest in larvae was 18 hours. Although in adults it had occurred earlier in the younger specimens than in older ones it was present in half of the cases in 10 days. By the fourth day it was observed in one-half of the larvae. About one-third of adult grafts and most of the larval eyes showed no corneal or lenticular opacity. In a few cases of adult and larval grafts the opacity was transient but in any case of excessive trauma sloughing of the bulb was subsequent to a

² Waltner, K., and Waltner, K., *Klin. Wochenschr.*, 1929, **8**, 313.

corneal change. In the black pigmented iris of adult grafts changes were dependent upon early return of circulation. When they took place a subcorneal hemorrhage involving a segment of the iris appeared during the first 2 weeks. If limited in area regeneration was complete within a month in which a new capillary network was formed. If extensive, but not involving sloughing, it was accompanied by a delay in retinal regeneration. In the yellow pigmented larval eye a slight temporary dulling of the luster of the iris sometimes occurred during the first 2 or 3 weeks. It was frequently accompanied by a subcorneal hemorrhage.

A slight postoperative reduction in the size of adult and larval grafts is usually found during degenerative changes in the retina. In the growing larval eye it is more marked until circulation returns¹ and by the twentieth day a size equal to that of the normal control is usually obtained. In adult grafts the size reduction is usually permanently established by about a month as in *Triturus*² or slightly longer depending upon the age of the host and the amount of degeneration. In adult grafts miosis was noted in some cases immediately after operation—a condition which persisted only about a week. In larval grafts immediately after operation miosis was observed in some and mydriasis in others, but the condition lasted only a few days. In many the pupil did not change. The pupillary reflex persisted uninterruptedly until death at 57 days in one young adult transplant. Usually the pupillary reflex was interrupted during the first week. This was followed by a recovery within 2 to 4 weeks. A few young adults showed an immediate postoperative absence of pupillary response lasting over several weeks before recovery. In larval grafts the pupillary reflex always occurred at metamorphosis and continued uninterruptedly.^{1, 3}

In larval grafts the retinal change is confined to a permanent loss of varying amounts of inner ganglion cells during a short period when the graft is temporarily decreased in size and a crowding of the retinal cell takes place. In adult grafts vast degeneration of all layers of the retina took place during the first 25 days extending to the ciliary margin. By about the thirty-seventh day in young adults all layers of the retina were represented by a rapid regeneration largely from the ciliary margin and partly from islands of undegenerated retinal cells. Complete restoration in old adults was

¹ Stone, L. S., *J. Exp. Zool.*, 1930, **55**, 193.

² Stone, L. S., and Ussher, N. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, **25**, 213.

³ Beers, D. N., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 477.

retarded from 1 to 3 weeks. In adults the dégénération of the optic nerve accompanies that of the retina and is accomplished during the first month. During the second month the growth of the new optic nerve from the bulb accompanies resolution of the retina. The nerve frequently approaches normal in size and passes to the optic chiasma usually along a tortuous course. In only 2 cases did the optic nerve fail to leave the bulb by the end of the second month. In these cases the nerve travelled a short distance between the retina and the choroid. In larvae a complete degeneration of the optic nerve took place during the first week although retinal change was slight compared to adult grafts. By the end of the third week it was completely regenerated in all but 4 cases (3 were rotated about 180° , one was normally oriented). The optic nerve was usually tortuous and less than normal in size.

Regeneration in a young adult transplanted eye was so rapid that return of vision was proven as early as the thirty-eighth day. Usually this was not determined in adults until after the second month. In larvae the earliest tests for vision which gave positive results were made on the forty-eighth day (a transplant). This host was still in a larval stage. Vision was proven in all types of grafts and in many specimens by methods already published in detail.¹ In them the microscopic studies showed complete optic nerves. Ocular movements were present and when the hosts were in the adult stage corneal and pupillary reflexes were demonstrated.

5786

Experiments on the Development of the Hind Limb of *Amblystoma*.

WALTER ALVA STULTZ. (Introduced by R. G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

Experiments with the hind limb of *Amblystoma punctatum* were undertaken to compare its developmental behavior with that of the fore limb, as described by Harrison.¹

In normal development the hind limb does not appear as a definite bud until approximately $2\frac{1}{2}$ weeks later than the fore limb, so that it lies latent in the host for a much longer period after transplantation than does the fore limb bud. Furthermore, the 2 halves of the

¹ *J. Exp. Zool.*, 1921, **32**, 4.

pelvic girdle are intimately fused in the median line, while the corresponding halves of the pectoral girdle are separate.

A preliminary experiment was done to determine the location of the hind limb rudiment at the stages used for operation. During the tail bud stage it was found that it lies ventral to myotomes 16, 17 and 18. The posterior rounded part of the body wall is its approximate caudal limit.

The following experiments were performed to test the equipotentiality of the hind limb. The limb rudiment was removed and the wound allowed to heal without covering. In this case a complete limb and girdle developed from the surrounding tissue. The same result followed when only a part of the rudiment was removed and the wound left uncovered. When the limb rudiment was extirpated and the area blocked with indifferent ectoderm, no limb developed, and the pelvic girdle was absent. When all, or a sufficiently large part of the hind limb rudiment was transplanted to an abnormal location, such as the flank, a complete limb and girdle developed at the site of implantation.

Hence, the hind limb of *Amblystoma* constitutes a harmonic equipotential system, just as Harrison found to be the case with the fore limb.² The pelvic girdle, however, differs from the pectoral girdle in that it also constitutes a harmonic equipotential system, while the girdle of the fore limb has been shown by Detwiler to be a mosaic system.³

To ascertain the symmetry relations of the hind limb of *Amblystoma*, the 8 experiments made by Harrison upon the fore limb were made.⁴ That is, limb rudiments were transplanted both in normal and in abnormal locations, thus constituting orthotopic and heterotopic grafts respectively. In each, grafts were made both on the same side and on the opposite side of body to origin; the antero-posterior axis of the graft was oriented both normally and with 180° rotation. The medio-lateral axis was not varied.

The results obtained by the heterotopic transplantations show that when the antero-posterior axis of the limb disc is reversed in grafting, the resulting limb is always disharmonic, *i. e.*, it is of opposite laterality to the side on which it is placed; while it always develops harmonically (with the same laterality as the side on which it is placed) when the antero-posterior axis is directed normally.

The results obtained with the orthotopic transplantations show

² *J. Exp. Zool.*, 1918, **25**, 424.

³ *J. Exp. Zool.*, 1918, **25**, 523.

⁴ *J. Exp. Zool.*, 1921, **32**, 7.

that the limb rudiment always develops into a harmonic limb whether its antero-posterior axis is reversed or not in grafting.

Reduplications took place in a minor number of cases in both orthotopic and heterotopic experiments. In the heterotopic operations they occurred only after 2 of the 4 orientations, namely, after transplantation to the same side of the body as that of origin, with the antero-posterior axis reversed, and after transplantation to the side of the body opposite its origin with the antero-posterior axis not reversed. That is, reduplications took place only in cases where the dorso-ventral axis had been inverted. In the orthotopic series reduplications occurred in most cases only after reversal of the antero-posterior axis of the graft. In both heterotopic and orthotopic grafts the primary member of the reduplicated limbs had the same laterality as a single limb would have had in the same location. With a few heterotopic cases, however, in which the graft was very large, 2 limbs were formed in serial alignment.

It is seen that the heterotopic grafts follow the same rules as apply to the fore limb according to Harrison's experiments.⁵ The orthotopic grafts, however, follow different rules in that the reversal of the antero-posterior axis of the rudiment does not prevent the harmonic development of the limb, just as occurs when this axis is not reversed. The result with orthotopic grafts shows that the regulatory influence of the normal position of implantation predominates over the axial polarization which exists in the limb rudiment at the time of transplantation. It is possible that this regulatory influence may be due to the length of time during which the hind limb rudiment lies latent in the host. As an alternative explanation it is also possible that the intimate connection between the two halves of the hind limb girdle may exert an influence in producing harmonic limbs.

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Innervation and Function of Grafted Hind Limbs in *Amblystoma Punctatum*.

HARVEY B. LOVELL. (Introduced by S. R. Detwiler.)

From the Zoological Laboratory, Harvard University, and the Biological Laboratories, University of Louisville.

Experiments were carried out to study the effects of a heterotopic limb upon the growth of the spinal nerves in the vicinity of the lum-

⁵ *Roux. Arch. f. Entw. Mech.*, 1925, **106**, 497.

bosacral plexus and to analyze the muscular movements of the limbs.

The hind-limb rudiment of *Amblystoma punctatum* was excised at stages varying from 39 to 45 and implanted from one to 5 segments anterior to its normal position. Two types of operations were performed, an autoplastic series in which the right hind-limb rudiment was excised and reimplanted to the flank, and a homoplastic series in which either a right or a left hind-limb bud was transplanted to corresponding positions anterior to the normal limb.

The limb rudiments transplanted at stages 42 to 45 were given various orientations. It was found that both the antero-posterior axis and the dorsoventral axis are irreversibly fixed at stage 42.

When the wound made by the removal of the limb bud was covered with indifferent ectoderm from the flank region, regeneration occurred in only 9 out of 51 cases. When the wound was not cleaned or covered regeneration took place in 59 out of 96 cases.

The hind-limb is normally innervated by the ventral rami of the 15th, 16th, and 17th spinal nerves. Coordinate and homologous movements were observed in many grafted limbs which had been implanted from one to 3 segments anterior to their normal position. The type of function was similar to that reported by Weiss,¹ Detwiler,² and Detwiler and Carpenter.³ The homologous muscle groups in the heterotopic limb, irrespective of limb orientation, flexed and extended synchronously with those of the ipsilateral normal limb, and alternate with those of the contralateral limb. The grafted limbs in such cases were found to be innervated by one or more branches from the normal lumbosacral plexus.

No definite coordinated movements could be observed in grafted limbs which were supplied entirely by nerves anterior to the 15th. Several grafts innervated by the 15th spinal nerve, together with nerves anterior to it, failed to function synchronously. In these cases the limbs were located in the dorsal portion of the flank in the epaxial musculature and received only the enlarged dorsal ramus of the 15th spinal nerve. This ramus normally has no connection with limb musculature. Another type of function observed was a sharp flexion of the ankle, foot, and digits of the graft, synchronous with the movements of the jaw muscles during feeding. The normal limbs and the animal as a whole were stationary during these movements. These heterotopic limbs were found generally to be

¹ Weiss, Paul, *Arch. fa. Mikros. Anat. u. Entw.-Mech.*, 1924, **102**, 635.

² Detwiler, S. R., *J. Comp. Neur.*, 1925, **38**, 461.

³ Detwiler, S. R., and Carpenter, R. L., *J. Comp. Neur.*, 1929, **47**, 427.

innervated by nerves anterior to the lumbosacral plexus, but in 3 cases they received, in addition, a dorsal ramus from the 15th spinal nerve.

Whether functional or not, the grafted limbs received extensive innervations from the spinal nerves. In order to reach the limbs the nerves traversed abnormal pathways through the body musculature. When limbs were grafted high on the side of the body they became innervated by enlarged dorsal rami which grew laterally or dorso-laterally to reach them. Ventrally situated grafts were supplied by ventral rami which followed their normal paths until they reached the level of the limb and then turned abruptly laterally to enter them. Since the spinal nerves were already laid down at the time the operations were performed, it is probable that the ends of the nerves were cut, and that they regenerated into the graft as it differentiated.

The 2 chief nerves entering the thigh of a normal hind-limb are the cruralis and ischiadicus. By means of graphic reconstructions of the plexuses to grafted limbs both these nerves could be identified in their usual positions. This was true even for limbs whose antero-posterior axes had been reversed. At the base of the grafts, as in normal limbs, the ischiadicus communicated with the cruralis.

Cell counts of the spinal ganglia showed that there is extensive sensory hypoplasia following the removal of the hind-limb, and extensive hyperplasia in the ganglia supplying a supernumerary limb.

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Experiments on the Determination and Development of the Pronephros of *Amblystoma Punctatum*.

DORIS E. FALES. (Introduced by Ross G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

This investigation deals with two problems; first the time at which determination of the pronephros occurs and second, the question of the transplantability of this organ and its relation to the various problems of development and function.

Prospective pronephric mesoderm from embryos of early stages was transplanted to the flank region of animals in the early tail bud stages. When this method is used, the capacity to form pronephric tubules is determined at the stage of widely open neural folds. (stage 15). The tubular pattern of 2 nephrostomal canals and a

common tubule is also determined at this stage. Although, according to this type of transplantation, the determination of tubular pattern occurs in embryos of stage 15, the pronephros acquires its complete determination gradually during the period from stage 15 to the time of the closure of the neural folds, since heterotopic grafts from embryos in these stages never develop completely normal organs. Transplants of partially determined prospective pronephric tissue develop into normal structures in the orthotopic position while they do not do so in heterotopic positions on the flank. This shows that determinative influences are still present in the normal surroundings of embryos in the early tail bud stages.

Tissue from embryos in the late gastrula stage (stage 12) and older has been transplanted into the body cavity of older *Amblystoma* larvae according to the technique described by Holtfreter.¹ Preliminary examination of this material indicates that, according to this method, determination has been acquired by stage 12. If this is substantiated a marked discrepancy will exist between the time at which the pronephros appears to be determined according to the 2 different tests, since, from the standpoint of development there is a wide gap between the gastrula and neurula stages. Experiments are in progress to determine the cause of this difference in the reaction of the grafts to the new environment.

In the experiments on the development of transplanted pronephroi, embryos from the stage just after the closure of the neural folds (stage 19) to that in which the tail bud is beginning (stage 25) were used. The pronephros of one side was transplanted from its normal location to the region ventral to somites 5 to 9. The development of these heterotopic grafts varies with their position in the host. Grafts placed ventral to somites 5 and 6 are usually morphologically normal and may develop into physiologically normal organs which can take over the entire pronephric function of the larva after the removal of the normal organ of the opposite side. In such cases the transplants show hypertrophy comparable to that occurring in the remaining organ after unilateral pronephrectomy.²

Grafts placed ventral to somites 7, 8, and 9 show a retarded development in early stages. They may become morphologically complete but all parts are much smaller than normal in animals which have reached the stage at which feeding begins (stage 46). These

¹ Holtfreter, J., *Roux's Archiv. f. Entwickl. mech. d. Organ*, Bd., 1929, **117**, 421.

² Howland, R., *Proc. Nat. Acad. Sci.*, 1916, **2**, 231; *J. Exp. Zool.*, 1921, **32**, 355.

organs are incapable of taking over the complete pronephric function of the animal although an enlargement of the tubules after removal of the normal pronephros indicates a response to the increased functional demand.

The presence of the graft in the posterior position results in the formation of glomerular capillaries by the host in the region of the transplanted nephrostomes.

When prospective pronephric tissue is placed just posterior to the normal pronephric region the tissues forming the common tubule of the transplant and the normal pronephros unite to form a single structure but the nephrostomal canals develop independently. In such grafts a continuous glomerulus is found extending from the region of the anterior nephrostome of the normal pronephros to the posterior funnel of the graft. The surface of this structure is markedly increased although the total volume appears little greater than normal, as the glomerulus is longer in the anterior-posterior axis but shorter in the lateral direction.

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Muscle Response to Foreign Innervation.

DONALD H. BARRON. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

Whether a muscle with foreign innervation will respond by contraction to any impulse reaching it or only to specific impulses which are characteristic for that muscle, has not yet been finally determined. To study the behavior of muscles with foreign innervation, anastomoses have been made between the individual nerve trunks of the brachial and lumbar plexuses in albino rats. The following anastomoses have been successfully obtained: sciatic to median and ulnar, sciatic to the radial, median and ulnar to the femoral, and the median with the ulnar to the sciatic.

The cut ends of the nerves were approximated and held in place by suturing with silk to the body wall. While so held the outgrowing fibers of the regenerating proximal nerve trunk grew along the distal trunk to the new muscle groups. A high percentage of the proximal nerve trunks showed evidence of regeneration and in about 50% of the 60 animals operated upon the nerves reached the muscle groups toward which they were directed.

By frequently reopening the incisions in the skin it was possible to locate the growing nerves and to follow the rate of regeneration of the outgrowing fibers. In young rats about 100 gm. in weight the daily outgrowth was about 3 mm. These nerves reached the muscle groups in about 40 to 45 days after the operation. The final length of the regenerated nerves was generally more than 3 times their original length. Observations made with and without ligation of the terminal stumps showed no cases which could be interpreted as a union by first intention, *i. e.*, Forsmann's suture.

The behavior of the muscle groups to which the foreign regenerating nerves were guided was studied by stimulating them electrically. Three or 4 days after the original nerve supply was cut off these muscles began to show the typical signs of atrophy. They decreased in size and gave the usual responses of degenerating muscle to galvanic and faradic stimulation. The arrival at these muscles of the outgrowing fibers of the regenerating nerve was followed by an increase in size, though they never completely recovered. The tone of the muscles also increased and when stimulated by galvanic and faradic currents they gave the responses of normal muscle. A stronger faradic shock, however, was required to cause contraction than was necessary for contraction of the normal control muscle.

Mechanical stimulation of the limb to which the outgrowing nerve fibers had been guided caused a reflex contraction in the original limb from which the nerve was drawn. This reflex was of the same type as might have been caused by the regenerated nerve in its original position. This type of observation was possible since the nerves of the brachial or the lumbar plexuses were divided in their distribution by the operation, some of the nerves being distributed to the fore limb and others to the hind limb. In the same way it has been possible to stimulate the limb in which the nerve was originally distributed and cause a contraction of the muscle groups with which it has established the new connection.

Thus it has been possible by means of simple mechanical stimulation applied to the peripheral sensory field of the original limb to cause impulses to pass over a nerve grafted to a new group of muscles. These impulses always cause contraction of the muscles which they reach. The muscles supplied, may be flexors or extensors, they respond as they would to impulses over their original nerve supply. So far there has been no indication of learning by these animals. The muscles are always contracted through reflex stimulation. These observations are interpreted as supporting the

experiments of Langley¹ which indicated that the result produced by impulses traveling in a nerve depends on where the fibers end and not upon any specific differences in the impulses themselves.

5790

Blood Pressure and Intestinal Motor Response to Splanchnic Stimulation after Nicotine.

MICHAEL G. MULINOS.

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Small intravenous doses of nicotine (1 to 5 mg. per kilo) depress the activity of the intestine, and abolish the effect of electrical stimulation of the vagus on the gut of the dog and cat.^{1, 2, 3} On the other hand, larger doses of nicotine (15 to 60 mg. per kilo) produce a stimulation of the intestine, and cause a return of the stimulatory effect of vagus faradization on the intestine.^{2, 3} These latter effects of nicotine are not due to an action on the splanchnic ganglia, or on the adrenal glands, for these effects are retained after the extirpation of both the ganglia and the adrenal glands.³

In this report the effects of intravenous injections of nicotine are extended to the activity of the splanchnic nerve in the cat. The animal is pithed and prepared as in the previous communication.³

The motility of the intestine was studied by a technic different from that reported in a previous communication.³ The abdominal wall is opened and the intestines are flooded with warm mineral oil. This minimizes distension due to loss of carbon dioxide.⁴ Two superficial stitches are then taken into the muscularis of the intestine, about 1.5 cm. apart, and firmly tied. To each of the strings is now attached one of the levers of the Cushny myocardiograph. The free arm of the instrument is then connected with the writing lever. If the activity of the circular coat is to be recorded, the stitches are taken in the same transverse plane; if the longitudinal coat is being studied, the stitches are in the same longitudinal plane.

¹ Langley, J. N., *J. Physiol.*, 1898, **23**, 240.

² Bayliss, W., and Starling, E., *J. Physiol.*, 1899, **24**, 99.

³ Thomas, J. E., and Kuntz, A., *Am. J. Physiol.*, 1926, **76**, 598.

² Mulinos, M. G., *Proc. Soc. Exp. Biol. and Med.*, 1927, **25**, 49.

⁴ Henderson, Y., *Am. J. Physiol.*, 1909, **23**, 345.

When the splanchnic nerve is to be stimulated, a Sherrington guarded electrode is put on the right nerve retroperitoneally. The adrenals are ligatured or removed also retroperitoneally, in order not to tear the peritoneum. The left vagus nerve was put into a guarded electrode in the neck.

Results. (a) Vagus Stimulation. The results obtained with this technic confirmed those previously reported.³ Apparently because of the lesser injury to the intestine, the nicotine abolished the motility of the gut and lowered its tonus only temporarily, and not permanently, as previously reported.³ It was further determined that the effects of vagus stimulation on the circulation and slowing of the heart are quickly and permanently abolished even by small doses of nicotine (1 to 5 mg. per kilo).

(b) Electrical stimulation of the splanchnic nerve raises the blood pressure and depresses the intestine, even in the absence of the adrenal glands. Immediately following the intravenous injection of 5 mg. per kilo of nicotine, there is a temporary loss of splanchnic activity, both on the gut and on the blood pressure. Further injections of nicotine progressively lose their power to abolish the effect of splanchnic stimulation on the gut and blood pressure. The nicotine itself soon loses its power to raise the blood pressure. Doses of over 15 to 60 mg. per kilo cause a lowering of the blood pressure at a time when splanchnic stimulation produces a rise in blood pressure and a depression of the intestine as great as before any nicotine was administered. The results are the same, if the adrenal glands are ligated or removed prior to the administration of the nicotine.

At a time when the autonomic ganglia are saturated with nicotine, as shown by their lack of response to further dosage, conduction through the ganglia from the pre-ganglionic to the post-ganglionic path is as efficient as ever. Certain ganglia in the body (only those of Auerbach's plexus and of the splanchnic area have so far been studied) may be saturated with nicotine, in the sense that these structures are no longer stimulated by subsequent doses of nicotine; yet can they conduct impulses apparently as well as ever.

Embryonic Rat Tissue Survival.

J. S. NICHOLAS.

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Observations upon developing living mammalian foetuses have been restricted mainly to their movements.^{1, 2} Since earlier stages of development offer definite information concerning the mechanics of mammalian morphogenesis, the problem of embryonic survival under varied conditions is of utmost importance. With this in mind the following experiments have been performed.

The rat embryos (8 and 9 days of gestation age) were carefully dissected free from the uterus and the *decidua capsularis* removed. The embryonic membranes which are just forming at the latter stage were left intact. The embryos were placed in Ringer's, Locke's, and physiological saline solutions and their morphological reactions noted. They were removed from the solutions at various times and fixed for histological study.

While the survival time varied considerably, no signs of histolysis were apparent after 6 hours in any of the above solutions at 37.5°C. When kept at 24°C. in the same solutions there was no sign of disintegration 12 hours after removal. Twenty embryos were preserved at 6 and 12 hour periods. The 12-hour embryos at body temperature showed considerable disintegration. The embryo retains its external form up to 72 hours at 24°C. but its cellular structure becomes atypical and shows characteristic degenerative changes. No increase in cellular differentiation occurs.

To determine whether embryos would develop in the mother outside of the abdominal cavity, a subcutaneous pocket was made in the body wall in which the horn of the uterus was placed with its contained embryos (8-9 days of age). The vascular connections were left intact and passed through incisions in the body wall. At the time of operation the embryos were normally imbedded in the uterine mucosa and the specimens removed for study were of 8 days' gestation age. Twelve such cases were permitted to carry their young to term. All except one had a normal birth and the animals so born were viable. Three other cases were preserved at stages intermediate between the stage of operation and full term. All the fetuses (14) were perfectly normal in development.

¹ Swenson, E. A., *Anat. Rec.*, 1925, **80**.

² Hooker, D., and Nicholas, J. S., *J. Comp. Neur.*, 1930, **50**.

In the rat the period of egg segmentation occurs in the Fallopian tube. If the tube is separated from the uterus on the third day after fertilization and the uterine lumina are ligated, the segmenting eggs fall into the abdominal cavity where they may either degenerate or continue their development. In 35 experiments, 5 positive results were obtained. Since this means 5 individuals in a total calculated as 250, positive results can be expected in only about 2%. These develop to term, have no decidua, but have a placental mechanism which works through vessels imbedded in the mesenteries.

Attempts to secure development of embryos by transplanting to various locations within the abdomen were unsuccessful. For this reason an investigation of transplantation sites outside the abdomen were made. Eight and 9-day rat embryos were transplanted to the outside of the body wall, to the fascia of the femoral region, and to various subcutaneous areas. In a few cases positive results were indicated and the embryonic transplant was vascularized.

The region of the mammary gland forms the best site so far secured. The response to the transplant is shown by hyperemia in the region of the transplant. Since this response might be assumed to be due to the mechanical irritation superimposed upon the hyperemia normal to the mammary gland during the later stages of pregnancy, similar transplantations were made in young male rats, 80 gm. in weight. Here the possible influence of ovarian secretions as well as cyclic mammary hyperemia was eliminated. Eighty percent of the grafts so made were recovered (40 cases, 4 grafts each) from 5 to 10 days subsequent to transplantation. Serial sections through the grafts show differentiating embryonic tissues, definitely recognizable as tissue developed from the embryonic transplant.

The following tissues have so far been identified, differentiated gut including circular and longitudinal musculature along with the intestinal epithelium, cartilage, embryonic red blood cells, striated muscle, nervous tissue and placental cells. The grafts resemble those described by Murphy³ and Willier⁴ after transplanting tissues upon the chorioallantoic membrane of the chick.

The results so far obtained justify the following conclusions: (1) Rat embryos will withstand the handling necessary for experiment. (2) They will survive immersion in salt solutions for long periods without histolysis. (3) Extrauterine pregnancy may be brought about in a limited number of cases by cutting the uterine

³ Murphy, J. B., *J. Exp. Med.*, 1913, **17**.

⁴ Willier, B. H., *Am. J. Anat.*, 1924, **33**.

horn after fertilization and permitting the segmenting blastulae to fall into the abdominal cavity. (4) Differentiation and growth occur regularly in embryos after transposing the uterus from the abdominal cavity to a pocket under the skin outside the abdominal cavity. (5) When embryos (without decidua) are transplanted to the mammary gland, either of males or females, differentiation of tissues takes place. The tissues are not organized to form a whole embryo.

5792

Physiology of the Metabolism of the Purine Nucleosides.

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Upon feeding the purine nucleosides, guanosine and adenosine, to dogs maintained on a synthetic diet, we find amounts of 2 to 3 gm. almost completely metabolized.

In case of guanosine about one-half of the nitrogen is metabolized to allantoin. One-third of the nitrogen is accounted for by an increased output of urinary urea. This is more than could arise from the amino group of the guanosine alone.

In the case of adenosine, we find the same increase in urinary allantoin, but practically no increase in urinary urea or ammonia.

Our results seem to indicate that in the dog the end product of purine metabolism is not allantoin alone; part of the allantoin seems to be further broken down to urea.

The experiments were carried out on 4 dogs. For a description of the experimental technique we refer to our previous publications.¹

Experiments dealing with the metabolism of the purine bases, guanine and adenine, are in progress.

¹ Cerecedo, L. R., *J. Biol. Chem.*, 1931, **98**, 269.

5793

Effects of Anoxemia on Intrapleural Pressure in Dogs.*

MYRON PRINZMETAL, LESTER LONERGAN AND SELLING BRILL.

(Introduced by C. D. Leake.)

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Department of Surgery, University of California Medical School, San Francisco.*

The theory has been advanced that changes in intrapleural pressure following alterations in bronchial caliber are mediated by carbon dioxide.¹ Thus in broncho-constriction, a gradual increase in carbon dioxide tension in the alveoli and blood seems to stimulate the inspiratory mechanism so that a relatively increased average thoracic girth results, and intrapleural pressure becomes more negative. If, however, stimulation of the respiratory center, by the direct administration of relatively high concentrations of carbon dioxide, becomes great enough to initiate active expiratory effort, then the intrapleural pressure becomes more positive than usual during expiration.² Since oxygen want in itself is, as was shown by Gasser and Loevenhart,³ initially stimulating to the respiratory center, we thought it might be interesting to determine directly whether or not anoxemia would alter intrapleural pressure in the same way as increased carbon dioxide tensions.

Anoxemia was produced by rebreathing into a spirometer through a tracheal cannula. Carbon dioxide was absorbed by soda lime placed in the circuit. Gas analyses were made by means of a Henderson-Haldane-Bailey⁴ apparatus in order to determine the degree of anoxemia produced and also to determine whether or not carbon dioxide was adequately absorbed. Blood gas analyses were also made in certain experiments. Details of our technique for estimating intrapleural pressure have been previously described.⁵

Anoxemia produced by this method (18 experiments) was found uniformly to result in an initial gradual decrease in intrapleural

* Supported in part by the J. J. and Nettie Mack and the Purington Research Funds.

¹ Prinzmetal, M., Brill, S., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 836.

² Prinzmetal, M., Brill, S., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 832.

³ Gasser, H. S., and Loevenhart, A. S., *J. Pharmacol. Exp. Therap.*, 1914, **5**, 239.

⁴ Bailey, C. V., *J. Lab. Clin. Med.*, 1921, **1**, 657.

⁵ Brill, S., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 518.

The duration of anoxemia was seldom more than 4 minutes. Analysis of respiratory air showed that within this period carbon dioxide was absorbed and that oxygen tension (corrected) was progressively reduced to between 52 and 90 mm. of mercury. Blood gas analysis indicated marked anoxemia. After about 4 minutes of this gradually induced anoxemia a secondary increase in intrapleural pressure was usually observed, accompanied by a mean decrease in thoracic girth and marked slowing of respiration. This corresponds to Loevenhart's secondary depression of the respiratory center as a result of continued oxygen want. It is to be noted that anoxemia like excess carbon dioxide tension seems first to stimulate the inspiratory mechanism and then later that concerned with expiration.

The initial increase of thoracic girth with more negative intrapleural pressure under the influence of mild anoxemia offers an explanation of the mechanism by which emphysema develops during continued residence at high altitudes.

5794

Phenomenon of Local Skin Reactivity to Serum Precipitates.

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From the Laboratories of the Mount Sinai Hospital, New York.

Phenomenon of local skin reactivity to serum precipitates is elicited as follows:

The abdominal skin of a rabbit is injected with 0.25 cc. of undiluted *B. typhosus* "agar washings" filtrate.¹ Twenty-four hours later the rabbit receives a single intravenous injection of a suspension of serum precipitate prepared in the manner about to be described. Four to 5 hours after the intravenous injection there appears at the prepared skin site a severe hemorrhagic and necrotic lesion characteristic of the phenomenon of local skin reactivity to bacterial filtrates.²

The serum precipitates with which the phenomenon could be consistently reproduced were obtained from the following mixtures:

¹ Schwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 843.

² Schwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 560; *J. Exp. Med.*, 1928, **48**, 247.

1. Normal horse serum with anti-horse rabbit serum.
2. Antimeningococcus horse serum with anti-horse rabbit serum.
3. Antimeningococcus horse serum with anti-horse goat serum.
4. Antimeningococcus anti-human horse serum with normal human serum.

The mixtures were made in proportions yielding maximum amount of precipitate, incubated in water bath at 37° for 2 hours, kept in the refrigerator over night and centrifuged at high speed. The supernatant fluid was removed and the sediment taken up in 0.85% NaCl solution in volume equal to $\frac{1}{4}$ of the volume of horse serum used.

A dose of 1 cc. of the suspension per kilo of body weight injected intravenously elicited severe reactions in a high percentage of rabbits. The reacting potency could be easily titrated by testing various dilutions of a suspension. Each dilution was tested in a group of 3 rabbits. The titer usually varied between 1:5 and 1:50 dilutions. The toxicity of the serum precipitates was quite stable since almost identical results were obtained on retests after a lapse of several weeks and months.

There was no apparent relationship between the amount of precipitate obtained in various preparations and their reacting potency. Some abundant serum precipitates proved totally inactive, while other slightly turbid suspensions were highly potent.

Furthermore, for the same ingredients the reacting potency of a mixture did not depend on the amount of precipitate obtained in it. By employing different proportions of ingredients there were obtained gradations in the amount of precipitate. If with a certain proportion there was obtained maximum precipitation the precipitate was potent and the supernatant fluid inactive. However, if the ingredients were mixed in a proportion which gave only partial precipitation, then both the precipitate and the supernatant fluid proved potent. The potent supernatant fluid was usually clear at the time of the injection, and produced some precipitation in the refrigerator after several days. Thus, it was possible to obtain severe reactions with serum containing no visible precipitate as well as with serum showing abundant precipitate—both derived from mixtures of the same ingredients.

When a *B. typhosus* culture filtrate is mixed in the necessary proportions with an immune anti-typhoid horse serum, complete neutralization of *B. typhosus* reacting factors can be obtained.³ The mixtures usually form abundant precipitates. These precipitates

³ Shwartzman, G., *J. Exp. Med.*, 1931, **54**, 1.

treated in the same manner as the serum precipitates described above have no reacting potency.

In order to test whether whole sera by themselves or colloidal suspensions are capable of eliciting the reaction, the following substances were tested in rabbits prepared with *B. typhosus* "agar washings" filtrate and found totally devoid of reacting potency, namely:

Normal human, rabbit, horse, guinea pig, chicken and rat sera.

Immune rabbit, goat and horse sera.

Normal and immune chicken plasma.

Heparinized chicken plasma.

4% suspension of charcoal, 2% suspension of infusorial earth.

4% suspension of silicic acid and 10% gelatine.

According to Sickles⁴ reactions can be obtained by intravenous injection of agar into rabbits prepared with meningococcus toxic filtrate but not by intravenous injection of galactose, gelatin, serum and India ink. This observation is corroborated by the author of this paper.

Observations reported here seem to demonstrate liberation of a toxic principle from blood serum by production of some disturbance in its colloidal state. This principle is capable, then, of eliciting severe injury in a tissue made vulnerable by a bacterial filtrate. It remains to be seen whether the reacting potency of agar⁴ is due to some toxic principle carried by it or whether the agar liberates a toxic principle from the blood *in vivo*.

5795

On the Local Inhibition of the Shwartzman Phenomenon.

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If an intradermal injection of a potent bacterial filtrate is followed 24 hours later by an intravenous injection of the same or another potent bacterial filtrate, there occurs a hemorrhagic, necrotic lesion at the site of the preparatory skin injection, generally reaching its maximum 4 to 5 hours after the intravenous injection. This phenomenon was described by Shwartzman^{1, 2} and is known as the Shwartzman phenomenon.

⁴ Sickles, G. U., *J. Immun.*, 1931, **20**, 169.

* Moses Heineman Research Fellow.

¹ Shwartzman, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **25**, 560.

² Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 267.

A number of observations³ were reported by Schwartzman which indicate that the phenomenon is not anaphylactic in nature. On the contrary, it seems to represent a transient state of vulnerability of the prepared tissue which makes it highly susceptible to injurious factors present in the blood stream for a period of 4 to 48 hours after the preparatory injection.

Burnet⁴ was able to prevent the occurrence of the phenomenon in a portion of the prepared skin site by making a local injection of active material immediately before the intravenous injection. Because, in his experiments, toxin and toxoid were more effective than immune serum or saline, the inhibition was considered by him to have been due to local "desensitization". According to him, then, "the reaction is certainly not anaphylactic in any ordinary sense of the term, but it has some suggestive similarities to the classical anaphylactic phenomena." Before accepting his immunological explanation for the inhibition, it seemed necessary to determine whether the increased tension in the skin created by the second skin injection might mechanically collapse the local blood vessels and thus shield a portion of the prepared tissue from the injurious agents circulating in the blood stream. With this aim in view the following experiments were performed on domestic rabbits.

A. Five-tenths cc. of *Meningococcus* and *B. dysenteriae* (Shiga) "agar washings" filtrate, as described by Schwartzman,^{5, 6} were injected into the abdominal skin. Twenty-four hours later, from 0.15 to 0.20 cc. of the substances used to produce inhibition were injected into the center of the prepared skin sites. Immediately thereafter, 50 reacting units of meningococcus toxin were injected intravenously. The results were read 4 to 5 hours after the intravenous injection. The first skin injection elicited a variable degree of local edema. Consequently, the tension produced by the second skin injection varied, being less pronounced when the edema was more marked. There was recorded inhibition when a purpuric lesion occurred at the prepared skin site except for the area infiltrated by the second skin injection.† One could frequently prognosticate the

³ Schwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.

⁴ Burnet, F. M., *J. Path. and Bact.*, 1931, **34**, 45.

⁵ Schwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 843.

⁶ Schwartzman, G., *J. Inf. Dis.*, 1929, **45**, 232.

† At the site of inhibition there is an area of tissue relatively normal in appearance, which is surrounded by a zone of intense hemorrhage, necrosis, and polymorphonuclear infiltration, as in the histological picture of a Schwartzman reaction.²

elicitation of inhibition when the second intradermal injection produced a wheal with blanching of the skin.

TABLE I.

Skin Preparatory Factors	Inhibiting Substance	Intravenous Reacting Factors	No. of Rabbits Showing	
			Phenomenon	Inhibition
0.5 cc. meningococcus "agar washings" filtrate	Undil. pituitrin 0.2 cc.	50 units of meningococcus toxin	6	6
"	5% sterile egg white solution 0.1 cc.	"	3	2
"	Adrenalin (1-1000) 0.15 cc.	"	2	2
"	Meningococcus "agar washings" filtrate 0.2 cc.	"	4	1
"	Histamine (0.2 mgm.) 0.2 cc.	"	1	0
"	Normal NaCl 0.15 cc.	"	2	1
"	Formalinized (0.5%) NaCl 0.2 cc.	"	2	0
"	Phenolized (0.4%) NaCl 0.2 cc.	"	2	0
0.5 cc. <i>B. dysenteriae</i> (Shiga) "agar washings" filtrate	Normal human serum 0.2 cc.	"	3	2
"	Phenolized (0.4%) NaCl 0.2 cc.	"	2	2
"	Formalinized (0.5%) NaCl 0.2 cc.	"	2	2
"	Immune rabbit serum 0.2 cc.	"	3	3
"	Pituitrin, undil., 0.2 cc.	"	2	2
"	Pituitrin, dil. 1:3, 0.2 cc.	"	5	2
"	<i>B. dysenteriae</i> (Shiga) "agar washings" filtrate 0.2 cc.	"	2	2
"	Histamine (0.2 mgm.) 0.2 cc.	"	1	1
"	Adrenalin (1-1000) 0.2 cc.	"	2	1
"	Meningococcus "agar washings" filtrate 0.2 cc.	"	3	2

The results of these experiments are summarized in Table I. Only the rabbits which showed positive reactions are included, the negative ones being omitted from these protocols. It is possible that some of the latter were really negative because of complete inhibition. It is seen that, using *Meningococcus* and *B. dysenteriae* (Shiga) preparatory factors, local inhibition was produced by

adrenalin, pituitrin, normal saline, phenolized saline, formalinized saline, sterile egg white solution, immune rabbit serum, normal human serum, and *Meningococcus* and *B. dysenteriae* (Shiga) toxins. The most marked and constant inhibition was produced by pituitrin.†

B. A band of the abdominal skin was infiltrated with 1 cc. of *Meningococcus* or *B. dysenteriae* (Shiga) "agar washings" filtrate. Twenty-four hours later, 3 injections of 0.15 cc. of normal saline, or one of these toxins, or combinations of these were made into different areas of the same prepared skin site. Immediately thereafter, 50 reacting units of *Meningococcus* toxin were injected intravenously. In these experiments, from 1 to 3 areas of inhibition were produced. Inhibition seemed best obtained at the ends of the band infiltrated with preparatory factors. This may have been due to the fact that there was least toxin and least edema at the periphery of the prepared skin site. The results in this type of experiment are not so clear-cut as they are when only one inhibiting agent is used, as in A.

C. Five-tenths cc. of *Meningococcus* "agar washings" filtrate were injected into the abdominal skin. Twenty-four hours later, padded clamps, 5 mm. wide at the edge, were applied to the centers of the prepared skin sites and left attached for 60 minutes. Immediately after the application of the clamps, 50 reacting units of *Meningococcus* toxin were injected intravenously. The clamps, themselves, caused no apparent injury to the skin. Of 6 rabbits tested, only 2 showed reactions and in each there was definite inhibition at the site of attachment of the clamps.

Conclusions. Local inhibition of the Schwartzman phenomenon can be produced by injecting into the prepared skin site a number of various substances immediately before the intravenous injection of the reacting factors. "Desensitization" can also be obtained by clamping off a portion of the prepared area for one hour after the intravenous injection. It becomes evident, therefore, that the inhibition described by Burnet cannot be considered as anaphylactic desensitization. It seems also of interest to point out that Burnet in his own experiments did not observe any specificity of inhibition because, as he says, "Meningococcal material can desensitize a typhoid reaction and *vice versa*." In one of the experiments cited even saline effected a weak "desensitization". It seems more likely that the inhibition is due to diminished blood supply to the prepared

† Pituitrin, injected alone, often causes intense blanching of the injected area with a peripheral band of purpura.

area at the time of introduction of reacting factors into the blood stream.

Summary. A number of experiments are described which demonstrate that local inhibition of the Shwartzman phenomenon can be achieved by treating the prepared skin area with a variety of substances immediately prior to the intravenous injection of reacting factors or by clamping off a portion of the prepared skin area for one hour after the intravenous injection. It is believed that these agents produce their effects by creating a local ischemia which shields the prepared skin tissue from the injurious agents circulating in the blood stream.

5796

Effects of Diet and Fasting on Plasma Phosphatase.

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Increased plasma phosphatase has been reported by Kay¹ and others in v. Recklinghausen's disease, in Paget's disease, in active rickets and in other pathological bone conditions. These results were confirmed by ourselves in our clinical material. Kay suggested that increased plasma phosphatase in these conditions was due to leakage of the enzyme from bone at more than the normal rate. However, in view of the presence of large amounts of phosphatase in other tissues than bone (kidneys, intestinal mucosa) it seems that variations in plasma phosphatase might be due to other causes, and that the state of nutrition of an animal might influence the plasma phosphatase in bone diseases found clinically or produced experimentally. The latter possibility had to be considered by us in studies of plasma phosphatase changes in experimental hyperparathyroidism, in view of the diminished appetite which is observed in animals treated with parathormone for long periods.^{2, 3} (In other experimentally produced conditions asso-

¹ Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

² Jaffe, H. L., and Bodansky, A., *J. Exp. Med.*, 1930, **52**, 669; Jaffe, H. L., Bodansky, A., and Blair, J. E., *Arch. Path.*, 1931, **11**, 207.

³ Bodansky, A., Jaffe, H. L., and Blair, J. E., *J. Biol. Chem.*, 1930, **88**, 629; Bodansky, A., and Jaffe, H. L., *J. Exp. Med.*, 1931, **53**, 591.

ciated with anorexia, malnutrition, stationary weight and loss of weight, the same possibility exists.)

In a preliminary study of phosphatase variations in experimental hyperparathyroidism in guinea pigs⁴ it appeared indeed that fasted control animals of the same age and kept under the same conditions as fed controls and as the hyperparathyroid animals showed a lower plasma phosphatase than the fed controls. Three fasted controls showed plasma phosphatase values of 3.9, 7.5, and 5.6 units per 100 cc. (*average* 5.7). Two fed controls showed plasma phosphatase of 8.7 and 11.3 units per 100 cc. (*average* 10.0). The data on a group so small required confirmation.

The blood was drawn from the heart in the guinea pigs and rats, and from the jugular vein in the dogs. Oxalated plasma was used for the determination of the phosphatase. We adhered to the use of oxalated plasma because our earlier data were obtained on oxalated plasma. It may be noted, however, that oxalate, in the proportions used to prevent coagulation, inhibits phosphatase activity. Heparinized plasma and serum show higher phosphatase content than oxalated plasma. Serum treated with like amounts of oxalate shows a phosphatase activity similar to that of oxalated plasma and about 10% lower than serum not so treated.⁵ We reduced the incubation period to 1 hour because of the high phosphatase content of our material. In other respects the details of our method were followed, as previously published.⁶

Effect of fasting on plasma phosphatase in guinea pigs. Two groups of young guinea pigs in good state of nutrition and weighing approximately 300-400 gm. were tested at 2 different times. In each of these groups the animals to be fasted were kept together with the others until the removal of food. We found it desirable to "condition" our animals alike before the tests. The results are shown in Table I.

Influence of the diet on plasma phosphatase in rats. Several litters of young rats were so divided that animals from each litter were represented in a group receiving the Sherman diet and in another group receiving a diet of ground fresh lean meat supplemented with calcium carbonate, cod liver oil, and tomato juice. The weights of these rats ranged from 60-105 gm. at the beginning of the experiment. Most of them gained during the following month 100-150%

⁴ Bodansky, A., and Jaffe, H. L., *Proc. Am. Soc. Biol. Chem.*, Montreal, Canada, April 8-11; *J. Biol. Chem.*, 1931, **92**, 16.

⁵ Bodansky, A., to be published.

⁶ Bodansky, A., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 760.

TABLE I.
Effect of Fasting on Plasma Phosphatase in Guinea Pigs.
 Plasma phosphatase content is stated in units per 100 cc.

		No. of Animals	Phosphatase		
			Max.	Min.	Av.
Group 1	Fed	5	12.9	6.3	9.1
	Fasted 3 days	4	7.4	2.8	5.1
Group 2	Fed	4	12.5	11.2	11.7
	Fasted 3 "	3	7.8	7.4	7.6
	" 6 "	3	4.0	1.7	2.6

of their initial weight. The average plasma phosphatase of 8 rats receiving the meat diet was *87.1 units per 100 cc.* (maximum 106.2, minimum 65.0 units). The average plasma phosphatase of 7 rats receiving the Sherman diet was *65.9 units per 100 cc.* (maximum 80.1, minimum 58.0 units). Two "runts" who, however, gained about 150% in weight on the meat diet showed an average plasma phosphatase of *84.5 units*, and 3 "runts" who gained equally on the Sherman diet showed an average plasma phosphatase of *52.9 units*.*

Effect of fasting on plasma phosphatase of rats. Five rats from both groups in the above test were fasted for 96 hours. Their plasma phosphatase averaged *26.8 units per 100 cc.* (maximum 38.1, minimum 20.0 units).

Dogs. Our data were less conclusive, probably because the fasting periods were shorter (60 hours). Incomplete results indicate, however, that after about a week of stationary weight the plasma phosphatase falls, and that a period of good nutrition restores the plasma phosphatase to a higher level. Seven puppies (litter mates), about 2 months old at the beginning of the tests, were used.

Summary and Conclusions. Rats maintained for over a month on the Sherman diet showed a lower plasma phosphatase than those maintained on a meat diet. The plasma of fasted rats and guinea pigs had a much lower phosphatase content than the plasma of fed controls.

The variations of plasma phosphatase could not in these cases be attributed to any phenomena associated with the physical and chemical processes taking place in bone. Indeed, the assumption that plasma phosphatase changes are caused only by physical or chemical changes in the bone would lead to expect results contrary to those we actually obtained, because starvation for 3 to 6 days

* We consider it important to note in this connection that these rats were maintained in like conditions in our laboratory for over a month. A litter of young rats obtained from another source and used immediately, without being "conditioned" in our laboratory, showed an average plasma phosphatase of less than 20.0 units.

causes considerable non-specific bone resorption.⁷ It seemed more plausible that the physical and chemical processes associated with nutrition were closely linked with these changes in plasma phosphatase. It is possible, however, that the cessation of new bone formation in young animals (also observed during starvation) contributes to the lowering of plasma phosphatase. It must be noted in this connection that young, growing animals were used, and that our conclusions do not necessarily apply to adults.

5797

Viosterol Treatment in Experimental Hyperparathyroidism.*

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Irradiated ergosterol (viosterol) has been employed therapeutically in clinical hyperparathyroidism (*Ostitis fibrosa cystica*, or v. Recklinghausen's disease) to supplement operative treatment. Snapper¹ referred to one case in which "vigantol" had to be given to promote the recalcification of the skeleton after removal of a parathyroid adenoma; he also mentions Regnier's mild case of clinical hyperparathyroidism in which considerable amelioration of the condition had been induced with "vigantol", ultraviolet light, and heat. However, Wilder and Johnson² stated that viosterol did not prevent the decalcification of the skeleton in experimental hyperparathyroidism in rats.

The protective and curative effects of small doses of irradiated ergosterol in disorders of calcium metabolism are well known, especially through its use in rickets. On the other hand, large and toxic doses may induce decalcification of the skeleton and a negative mineral balance (Shohl, Goldblatt and Brown,³ Soeur,⁴ and Taylor, Weld, Branion, and Kay.⁵)

⁷ Jaffe, H. L., and Bodansky, A., unpublished results.

* The generous cooperation of Eli Lilly and Company, who supplied most of the parathormone used in these experiments, is acknowledged.

¹ Snapper, I., *Ann. de Méd.*, 1931, **20**, 201.

² Wilder, R. M., Johnson, J. L., *Proc. Assn. Am. Phys.*, abstracted in *J. Am. Med. Assn.*, 1931, **96**, 1987.

³ Shohl, A. T., Goldblatt, H., Brown, H. B., *J. Clin. Invest.*, 1930, **8**, 505.

⁴ Soeur, R., *La Presse Méd.*, 1931, **53**, 1003, and *Arch. Int. de Méd. Expér.*, 1931, **6**, 365.

⁵ Taylor, N. B., Weld, C. B., Branion, H. D., Kay, H. D., *J. Can. Med. Assn.*, 1931, **25**, 20.

Rapid decalcification and fibrous repair of bone in guinea pigs treated with suitably high doses of parathormone has been observed regularly.^{6, 7, 8, 9, 10} In this study we investigated the possible influence of viosterol on the bone lesions of experimental hyperparathyroidism. The viosterol (Mead Johnson and Co. 250-D, diluted with corn oil) was given by mouth. A tuberculin syringe with a 19 gauge needle with squared end was used to allow accurate measurement of the doses.

The experimental animals were divided into 3 groups. The first group consisted of 5 guinea pigs weighing between 220 and 290 gm. They were first given viosterol daily for 7 to 10 days. The daily dose of viosterol was adjusted so that one guinea pig of the group received 10 mg. (250-D), 2 received 20 mg., and 1 each received 40 mg. and 80 mg., respectively. After the preliminary treatment with viosterol, the animals were injected daily with parathormone in increasing amounts, and the viosterol was continued. Beginning with 4 units of parathormone, the dose was gradually increased to 40 units, each guinea pig receiving a total of 530 units over a period of 23 days.

The second group of 5 animals weighed from 222 to 352 gm. The viosterol doses were the same as for Group 1, but the injections of parathormone were started simultaneously with the viosterol treatment. In this group the administration of viosterol and parathormone extended over a period of 29 days. As before, the dosage of parathormone was gradually increased from 4 units to 40 units daily. Each animal received a total of 575 units.

The third group contained 6 controls weighing between 210 and 300 gm., injected with parathormone as the guinea pigs in Group 2. *These control animals did not receive viosterol.*

The guinea pigs in all 3 groups were killed to terminate the experiment, and their bones were taken for histological examination. These showed decalcification and secondary fibrous invasion of the bones, as previously described. There was no consistent difference as to the nature and severity of the lesions between the guinea pigs receiving viosterol and parathormone (Groups 1 and 2) and those receiving parathormone only.

Under the conditions of this experiment irradiated ergosterol did

⁶ Jaffe, H. L., Bodansky, A., Blair, J. E., *Arch. Path.*, 1931, **11**, 207.

⁷ Bodansky, A., Blair, J. E., Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

⁸ Jaffe, H. L., Bodansky, A., Blair, J. E., *J. Exp. Med.*, in press.

⁹ Jaffe, H. L., Bodansky, A., Blair, J. E., *Arch. Path.*, in press; *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 793.

¹⁰ Bodansky, A., Jaffe, H. L., *J. Biol. Chem.*, 1931, **93**, 543.

not protect the guinea pigs from the demineralizing effects of experimental hyperparathyroidism. It is possible, however, that the healing of bone lesions in experimental or clinical hyperparathyroidism would still be promoted by viosterol after the state of hyperparathyroidism had been terminated by discontinuance of parathormone administration in experimental animals, or by the removal of a parathyroid adenoma in clinical cases.

5798

Effects of Hypophyseal Extracts on Sexually Immature Monkeys.*

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Information concerning the effects of anterior pituitary materials on the gonads of primates is very limited. E. Allen¹ and Hartman² have demonstrated the gonad stimulating effect of hypophyseal implants using dogs, monkeys, and pigs as donors, and Courrier, *et al.*,³ have shown that similar effects can be obtained with alkaline hypophyseal extracts. We wish to report the results of injecting aqueous pyridine extracts of anterior pituitary material in immature female *Macacus rhesus* monkeys.†

Aqueous pyridine extracts as prepared by Fevold, *et al.*,⁴ were used. The preparations were injected subcutaneously in 0.5 cc. doses twice daily for periods of 14 to 16 days and each cc. of extract was equivalent to 1 gm. of dried pituitary powder. As small a dose as 8 gm. equivalent of pituitary was found to be sufficient in one animal. The first noticeable change observed was the appearance, in 2 to 3 days of injection period, of a purplish coloration in the skin of the peri-anal region and a reddening of the nipples of the mammae. The maximum coloration and peri-anal swelling was reached in about 8-12 days, the vaginal smear at this time showing predominantly cornified cells. When the injections are

* Aided in part by grants from the National Research Council, Committee on Problems of Sex.

¹ Allen, E., *Anat. Rec.*, 1928, **39**, 315.

² Hartman, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 338.

³ Courrier, R., Kehl, R., Raynaud, R., *C. R. S. de Biol.*, 1929, **101**, 1093.

† The pituitary powder was kindly furnished by the Research Laboratories of Parke, Davis and Company.

⁴ Fevold, H. L., Hisaw, F. L., Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 291.

continued further the coloration decreases somewhat but the oedematous condition in the inguinal region and the posterior part of the thigh becomes progressively greater. No blood could be detected in the vagina although uterine bleeding (follicular menstruation) followed the cessation of injections.

The effects of the treatment on the internal sexual organs were very marked. The ovaries increased in weight as much as 1800%, the increase being due to development of a number of large follicles. Corpora lutea were entirely absent except in one doubtful case where there seemed to be slight luteinization. Pyridine extracts, which produced both follicular and corpus luteum development in rats, stimulated only follicular development in monkeys, and these ovaries could not be luteinized when such treatment was followed by intraperitoneal injections of strong luteinizing fractions but instead the ovaries retrogressed to the immature state during which menstruation occurred. The uterine endometrium in no case showed corpus luteum effects but was of the type characteristic of follicular activity which can be duplicated in castrates by administering follicular hormone. If these animals are castrated after anterior lobe treatment and given corpus luteum hormone (corporin) an endometrium of the corpus luteum type is developed.

No instances of ovulation were observed nor could it be induced in the stimulated ovaries by intravenous injections of from 400 to 800 times the amount of anterior lobe extract necessary to elicit ovulation in rabbits.

When immature monkeys were given pituitary extracts, as described above, there was a marked growth of the mammary glands but lactation did not occur during or after cessation of treatment. In those cases, however, where anterior lobe treatment was followed by castration and injections of corpus luteum extracts lactation was quite pronounced.

Dried pyridine extracts put up in gelatin capsules and inserted into the vagina of an immature monkey in daily doses equivalent to 1 gm. of dried pituitary powder, for 9 days produced no noticeable vaginal or sexual skin changes.

Monkeys weighing 3000 to 3500 gm. apparently respond more readily and give a more exaggerated picture than those weighing about 1800 gm. This is presumably due to a difference in age though all animals used were sexually immature.

It seems, from these results, that monkeys may differ from laboratory rodents in their response to anterior lobe extracts with regard to development of lutein tissue and perhaps ovulation, otherwise

they give the same fundamental reactions known for other mammals. Yet these differences may be only apparent and due to some factor not controlled in the experiments.

5799

New Studies on the Filtrability of the Tubercle Group.

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Pittsburgh, Pa.*

A grass bacillus indistinguishable in the characteristic cultural and morphological earmarks from the tubercle bacillus was isolated 6 years ago. Its acid fastness was variable even in cultures isolated from a single cell. Under certain conditions it developed non-acid-fast granules which were filtered through a Berkefeld N candle. These underwent slow and capricious germination to a non-acid-fast, diphtheroidal bacillus whose cultural characters were quite different from the antecedent strain. Microscopically, however, the strains were almost indistinguishable.

In 4 out of 6 years of the intervening period, and always in the spring of the year, the identical diphtheroid has spontaneously dissociated from the original strain, indicating that the mechanism for such dissociation is *via* the filtrable gonidial granule. (Much.)

More recently, by employing a modified K-medium (Kendall) these observations have been repeated and extended; for in addition to the diphtheroidal form, the original cultural stage of the organism has been recovered from the filtrates. A strain of bovine tubercle bacillus has also dissociated a very similar diphtheroidal strain, often containing numerous acid-fast granules.

These strains differ in no essential particular from the diphtheroids of Hodgkin's disease, benign lymphogranulomatosis and Boeck's sarcoid, making it highly probable that their origin *in vivo* is also the tubercle bacillus. No opinion is vouchsafed in respect of their rôle in the pathogenesis of such diseases.

For almost 30 years non-acid-fast forms have been described for tubercle bacillus by various workers, but their mechanism of origin has never been clear and the strains themselves were viewed as contaminants.

This final demonstration that single-celled cultures of the acid-

fast group possess filtrable phases, biologically distinct, should settle this moot problem of the filtrability of acid-fast bacilli; it explains in addition the origin of certain non-acid-fast strains and may lead to their better evaluation in certain atypical forms of tuberculosis.

5800

Effect of Giving Digitalis on the Volume Output of the Heart and its Size in Normal Individuals.

HAROLD J. STEWART.

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It was first shown by Harrison and Leonard¹ that digitalis decreased the cardiac output in normal dogs. In repeating these experiments, Cohn and Stewart² were able to confirm the observation that digitalis decreased the volume output of blood from the heart in normal dogs, but that at the same time it *decreased* the size of the heart although it *increased* the extent of ventricular contraction. Results similar to these were obtained when the drug was given to dogs in which the hearts had become pathologically enlarged, but in which there were no signs of heart failure.³ In a parallel study, Burwell, Neighbors and Regen⁴ by an unsatisfactory method published data which suggested that taking digitalis decreased the cardiac output of normal men. Since the publication of their paper, Grollmann^{5, 6} has introduced a more accurate method of measuring the cardiac output than that formerly used. In the study here reported, this method has been used, which so far as can now be judged gives accurate and reliable results. The nature of the changes which Burwell, Neighbors and Regen found resemble those described in this report.

There are observations on 4 subjects. Digitan (Merck) 0.8 to 1.0 gm. was given in a single dose. Observations were made immediately before the drug was given and at frequent intervals afterward. All observations were made with subjects in a basal

¹ Harrison, T. R., and Leonard, B. W., *J. Clin. Invest.*, 1926, **3**, 1.

² Cohn, A. E., and Stewart, H. J., *J. Clin. Invest.*, 1928, **6**, 53.

³ Cohn, A. E., and Stewart, H. J., *J. Clin. Invest.*, 1928, **6**, 79.

⁴ Burwell, C. S., Neighbors, D. W., and Regen, E. M., *J. Clin. Invest.*, 1928, **5**, 125.

⁵ Grollmann, A., *Am. J. Physiol.*, 1929, **88**, 432.

⁶ Baumann, H., u. Grollmann, A., *Z. f. klin. Med.*, 1931, **115**, 41.

metabolic state. In addition to measurements of cardiac output, the size of the heart was measured in x-ray photographs taken at a distance of 2 meters. Electrocardiograms were made; the heart rate was counted; the blood pressure was estimated.

Records secured in studying one subject illustrate the course of events. The cardiac output before the administration of digitalis was 3.84 liters per minute. Two hours following the administration of digitan (Merck) 0.9 gm. the cardiac output remained unchanged; 4 hours afterward it had decreased to 3.68 liters per minute. Progressive decrease then occurred; 24 hours afterward the volume output measured 2.54 liters per minute, or 66% only of what it had been at the beginning. The cardiac output from this time returned slowly toward its initial value, although even at the end of 2 weeks, when it was 3.11 liters per minute, this had not been attained. The size of the heart decreased in a parallel fashion; 24 hours after the drug had been given its size had decreased from 105.8 sq. cm. to 97.7 sq. cm., that is to say, it measured 91% of its initial size. Although its size gradually increased, as in case of the cardiac output, the initial size had not been attained 2 weeks later, when it measured 102.8 sq. cm. The heart rate decreased following the administration of the drug. The electrocardiograms showed alterations in form of the T-wave. Curves showing this alteration will be published later. Both the systolic and diastolic blood pressure increased. At the time the cardiac output was lowest and the size of the heart smallest the subject experienced dyspnoea and cardiac pain on slight exertion.

The results in the 3 other cases are similar to those now reported, with this exception, that the effects of the drug in them was of shorter duration and passed off within 48 hours.

It seems clear, now that a reliable method of study is available, that with the administration of digitalis to normal men (1) the cardiac output *decreased*; the cardiac size *decreased*; (3) the cardiac rate *decreased*; (4) alterations of the T-wave of the electrocardiogram occurred; (5) the blood pressure was usually elevated; (6) the maximum effects were observed 9 to 24 hours after the drug had been given and had usually passed off at the end of 48 hours.

Effect of Giving Digitalis on the Volume Output of the Heart and its Size in Heart Failure.

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It appears to be a fact that the giving of digitalis decreases the cardiac output (Burwell, Neighbors and Regen,¹ and Stewart²) and cardiac size (Stewart³) in normal human beings. The effect of its administration when hearts are diseased is, however, still unknown. With the introduction by Grollman^{3,4} of the acetylene method for measuring the cardiac output in man, a method became available for studying this condition. In this investigation the method of Grollman has been used. In addition to estimations of the cardiac output, the size of the heart was measured from x-ray photographs taken at a distance of 2 meters. In addition to the usual hospital records, electrocardiograms were made and the heart rate counted. Blood pressure and vital capacity were also investigated, but not reported here. All observations were made with patients in a basal metabolic state, immediately before digitalis was given and at frequent intervals afterward.

The first patient, a male, 47 years of age, suffered from a first attack of heart failure. The records secured in studying his case illustrate the course of events in cases forming the group exhibiting normal cardiac mechanism and heart failure of the congestive type. The diagnosis was generalized arteriosclerosis, hypertension, rheumatic heart disease (mitral insufficiency), cardiac hypertrophy. His heart and liver were large. Ascites and edema of the lower extremities were present. The lungs were free of râles. During heart failure the cardiac output measured 2.88 liters per minute, *i. e.*, it was diminished, and the heart now 198.3 sq. cm. in area was larger than during the stage of compensation. On the first occasion that digitalis was given, diuresis occurred and the patient became free of the signs and symptoms of heart failure. On giving the drug (digitan 1.0 gm.) there occurred an immediate increase in the cardiac output to 3.54 liters per minute, decrease in size of the

¹ Burwell, C. S., Neighbors, D. W., and Regen, E. M., *J. Clin. Invest.*, 1928, **5**, 125.

² Stewart, H. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 207.

³ Grollman, A., *Am. J. Physiol.*, 1929, **88**, 432.

⁴ Baumann, H., u. Grollmann, A., *Z. f. klin. Med.*, 1931, **115**, 41.

heart to 180.2 sq. cm., slowing of the heart rate and alterations of the form of the T-wave of the electrocardiogram. As the effect of digitalis wore off, the signs of heart failure reappeared and 2 weeks later the cardiac output decreased to 2.62 liters per minute and the size of the heart increased to 193.6 sq. cm. When digitalis was given a second time (digitan 1.0 gm.) results similar to those first observed were obtained, that is to say, diuresis, increase in cardiac output and decrease in cardiac size. Later digitalis became ineffective for the relief of heart failure, for, although under its influence, cardiac output increased slightly and for a short time only; the corresponding changes in the size of the heart were slight. In each instance the maximum effect was observed 15 to 24 hours after the drug had been given.

A second patient, a male, 30 years of age, was suffering from a first attack of heart failure. The diagnosis was lues, chronic cardiac valvular disease (mitral insufficiency, aortic insufficiency) slight hypertension. He exhibited enlargement of the heart, a few râles at both lung bases posteriorly, small interlobar effusion (seen in the x-ray photograph), enlargement of the liver, ascites and edema of the lower extremities. Diuresis occurred on the administration of digitan (Merck) 1.0 gm. The cardiac output which had been 2.08 liters per minute, *i. e.*, less than normal, increased to 2.73 liters per minute and the cardiac size decreased to 130.4 sq. cm., from 143.4 sq. cm. As the effect of the drug wore off the symptoms and signs of heart failure recurred; 10 days later the cardiac output was 1.44 liters per minute only, *i. e.*, it had decreased, and the size of the heart was larger (134.2 sq. cm.). When digitan 1.0 gm. was given a second time diuresis failed to occur, but there was a moderate temporary rise in the cardiac output and decrease in the cardiac size. Theocalcin was then given. Diuresis occurred promptly and the patient became free of heart failure. There occurred in this instance also an immediate increase in cardiac output and decrease in cardiac size, these relations being maintained. It is not the intention at the present time to discuss the mechanism of theocalcin in such cases.

The effect of giving digitalis to patients suffering from auricular fibrillation was also studied. A woman, 50 years old, was suffering from a fourth attack of heart failure of the congestive type. The diagnosis was cardiac hypertrophy, chronic myocarditis, mitral insufficiency and auricular fibrillation. She exhibited a few râles at the lung bases posteriorly, edema of the lower extremities, ascites, enlargement of the liver and tachycardia. When digitan 1.0 gm

was given the ventricular rate became slower, the pulse deficit decreased, diuresis occurred and the patient became free of the signs of heart failure. The cardiac output which had been 2.44 liters per minute increased to 3.45 liters when digitalis was given and the cardiac size diminished to 167.3 sq. cm. from 185.3 sq. cm.

The effect of giving digitalis when the rhythm of the heart is normal and when there are no signs of congestive heart failure was shown in the case of a white girl 21 years old. She complained of dyspnoea. The diagnosis was rheumatic heart disease (mitral stenosis and insufficiency), and cardiac hypertrophy. The cardiac output increased from 2.19 to 3.00 liters per minute and the cardiac size diminished from 141.4 sq. cm. to 133.7 sq. cm. when digitan 1.0 gm. was given.

It appears, therefore, from the study of these cases that in the presence of heart failure (1) the cardiac output diminishes. (2) With the administration of digitalis (a) the cardiac output increases; (b) the cardiac size diminishes; (c) the ventricular rate decreases both when the rhythm is normal and in the presence of auricular fibrillation; (d) and alterations in the form of the T-wave of the electrocardiogram occur. (3) As the effect of digitalis wears off these functions change in the reverse direction.

Illinois Section.

Billings Hospital, November 3, 1931.

5802

Studies in Renal Denervation.* III. Bacterial Embolism in the Normal and Denervated Kidney.

RUDOLPH HECHT. (Introduced by W. F. Petersen.)

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College of Medicine.*

Medium sized rabbits were used throughout the experiment. The left kidney was denervated and the right kidney was left intact and used as a control. The rabbits were placed on normal diet.

The kidney was approached through a lumbar incision and denervated according to the method described by Milles, Müller and Petersen,¹ except that the animals were placed under ether anesthesia instead of Nembutal. Some of the animals were allowed to recover for 2 weeks; others for 2 months.

Five cc. of a concentrated suspension of an attenuated culture of *Staphylococcus aureus* in physiological salt solution was injected into the marginal ear vein of each rabbit. The fine clumps of bacterial growth were not broken up when removed from the agar plates and placed into the salt solution, in the hope that embolus formation would thus be favored. This was first tried on unoperated animals to determine the dosage necessary to produce emboli. It had been previously determined that this dosage would kill such rabbits in 4 to 5 days. It was then tried on denervated rabbits and the results indicated that the denervated kidneys contained about half as many emboli as the intact kidney.

The following rabbits were then used to make exact counts of

* The present investigation was aided by a grant from the Josiah Macy, Jr. Foundation.

¹ Milles, G., Müller, E. F., and Petersen, W. F., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 351.

the number of micotic abscesses formed. They were killed on the morning of the fourth day after the bacterial injection by injecting ether into the heart, the kidneys removed and fixed in formalin. Corresponding parts of the kidneys were sectioned serially and examined microscopically. The emboli were counted and the following results were obtained:

Two Week Recovery after sympathectomy of left kidney		Two Month Recovery after sympathectomy of left kidney	
Left	23 emboli	Left 1	24 emboli
Right	174 "	Right 1	72 "
		Left 2	13 "
		Right 2	36 "
		Left 3	120 "
		Right 3	263 "

Obviously the normal kidneys develop more micotic abscesses than the denervated kidneys.

The toxicity associated with the bacterial injection may act directly on the sympathetic nervous system or in the nerve endings of the arterioles or capillaries of the kidney with resulting contraction. This contraction on the normal side may impede the passage of small micotic emboli sufficiently to facilitate their retention in the glomeruli. The denervated kidney with its wider vascular bed and the absence of sympathetic nerve effects may offer freer passage for the emboli.² The slowing of the passage through the normal kidney as contrasted to the denervated kidney may permit greater opportunities for lodgement of the emboli in the tortuous capillaries of the glomeruli. These effects may be mediated through the shock effect of the injection and the consequent liberation of epinephrine with resulting action on the intact vasomotor system but having no influence on the denervated kidney.

The denervated kidney, because of its better blood supply may be able to destroy the bacteria that lodge in the kidney. Müller, Petersen and Rieder³ found that in the presence of a colon bacillæmia the intact kidney excreted not only large amounts of albumin, red blood corpuscles and casts but also bacteria in large numbers, while the denervated kidney remained impermeable. The experiments indicate the importance of the relative autonomic tonus in different organs for the lodgement of bacteria and the formation of emboli in bacteremia and sepsis.

² Milles, G., Müller, E. F., and Petersen, W. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 354, 561.

³ Müller, E. F., Petersen, W. F., and Rieder, W., *Verh. d. Deut. Gesell. f. inn. Med.*, 1930, **42**, 580.

5803

An Institutional Outbreak of Food Poisoning Possibly Due to a *Streptococcus*.*

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Streptococci have been found in foods implicated in food poisoning outbreaks,^{1, 2} but the evidence that these organisms were the etiologic agent rests only upon animal feeding experiments.¹ In the outbreak here reported green hemolytic streptococci were isolated from the sausage epidemiologically implicated. Green hemolytic streptococci were also isolated from an unopened can of sausage from the same lot as that involved in the outbreak, and this sausage, when eaten by a human volunteer, produced symptoms of food poisoning.

The outbreak involved 75 out of 182 boys who had eaten a Sunday night meal at which Vienna sausage had been served. The other foods eaten by the boys could be ruled out on epidemiological grounds, and the water supply, from deep artesian wells, was not implicated. The sausages, taken from 6 large (No. 10) cans, were mixed in a container and served without heating. No abnormal appearance, odor or taste was noted. It seems probable, from the fact that less than half of the boys became ill, that the contents of only one or two of the cans were contaminated.

Nausea, vomiting, colicky pains and diarrhea developed in most cases in 4 or 5 hours, but several of the boys were not ill until 12 hours after the supper. All recovered within 24 hours of onset of symptoms.

Three sausages left over from the supper were examined in our laboratory. Two of these contained large numbers of slowly growing, green, pleomorphic streptococci. The organisms were killed by heating to 60°C. for one hour and to 85° for 15 minutes. No organisms of the *Salmonella* group were found when Endo and eosin methylene blue plates were streaked with the sausage. Four unopened cans of sausage with the same code marking were later examined. One of these contained viable organisms almost identical with those isolated from the 2 sausages of the outbreak. The un-

* This study was aided by a grant from the National Cannery Association.

¹ Linden, B. A., Turner, W. R., and Thom, Charles, *U. S. Pub. Health Rpts.*, 1926, **41**, 1647.

² Nelson, N. A., *New England J. Med.*, 1928, **100**, 1145.

opened can in which the contaminated sausages were found showed defective seams through which the organisms might have gained entrance.

When cultures from the unopened can had been incubated for 4 days, but before growth was evident, a young adult laboratory worker ate half a sausage from each of the 4 cans. Five hours later she developed nausea which increased for 24 hours with 2 periods of vomiting and epigastric pain. Marked exhaustion occurred, followed by constipation. A second volunteer drank 40 cc. of a Berkefeld filtrate of a 5-day beef-heart culture and became ill 5 hours later with belching; nausea appeared 24 hours later, accompanied by unsuccessful attempts at vomiting, intermittent epigastric pain, tenesmus, cold perspiration, a temperature of 99.2°F., and marked exhaustion. Neither of the 2 volunteers was ordinarily subject to gastro-intestinal upsets. From a cultural test of the filtrate drunk by the second volunteer it was estimated that 10,800 living streptococci were present in the amount of filtrate consumed. A third volunteer drank 50 cc. of a bacteria-free filtrate in which the organisms had been grown, and suffered no ill effects.

Mice, guinea pigs, rabbits and monkeys (*Macacus rhesus*) were fed cultures of these streptococci, but showed no definite symptoms. Three young cats that were fed 48-hour milk cultures of the organisms had soft stools the following day, the stools becoming normal and formed again on the second or third day.

The strains isolated from the sausages served at the school reduced litmus milk, causing soft coagulation with a red ring at the surface. Acid was produced in mannite broth. The strain isolated from the unopened can slightly reduced litmus milk, with no coagulation. No acid was produced in mannite broth. The strains were alike in that gelatin was not liquefied. Dextrose, saccharose, lactose, galactose and salicin were fermented with acid but no gas. Dulcitol, inulin and xylose were not fermented.

We feel that the illness of the 75 boys was possibly due to living pleomorphic streptococci in the sausage, and that similar organisms isolated from an unopened can of the same lot were capable of producing similar symptoms in human volunteers. The sterile filtrate apparently was incapable of producing illness:

The Toxicity of the Products of Pancreatic and Gastric Digestion.*

H. E. HAYMOND AND LESTER R. DRAGSTEDT.

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The most widely accepted theory regarding the etiology and pathogenesis of acute pancreatitis may be briefly stated as follows: A premature or intra-pancreatic activation of the trypsinogen of pancreatic juice is caused by the entrance of bile or duodenal juice into the pancreatic ducts or as a result of trauma or infection. It is then further assumed that the active trypsin so produced digests away the gland, that the end products of this tryptic digestion of pancreas are toxic, that they are absorbed into the blood stream and occasion a fatal toxemia. Ellis and Dragstedt¹ found that the uncontaminated liver of normal dogs contains bacteria in a very high percentage of cases and that the toxemia resulting from liver autolysis *in vivo* is dependent upon the activity of these bacteria in the presence of necrotic liver. By a similar technique to be reported in detail elsewhere we have found that the normal uncontaminated pancreas of healthy adult dogs also regularly contains bacteria and that the fatal toxemia resulting from the intraperitoneal autolysis of pancreas is likewise in large measure dependent upon the activity of these bacteria in the presence of necrotic pancreas. In this connection it has occurred to us that a reinvestigation of the toxicity of gastric and pancreatic juice and of the end products of the disintegration of body tissue produced by these juices in the strict absence of bacterial activity would be desirable. The present experiments were designed to determine the toxicity of the end products of gastric and pancreatic digestion.

Gastric juice was obtained from a Pavlov pouch and fresh active pancreatic juice from a specially devised fistula, previously described.² These were sterilized by passage through a Berkefeld filter (N). Carefully weighed samples of dog pancreas, liver, and muscle were then autoclaved at 15 lbs. for 15 minutes. Proportionate amounts of the sterile juices were added to each and digestion allowed to proceed at 37°C. for 66 hours. A control experi-

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Ellis, J. C., and Dragstedt, L. R., *Arch. Surg.*, 1930, **20**, 8.

² Dragstedt, L. R., Montgomery, M. L., and Ellis, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 109.

ment was carried out with fresh uncontaminated, but not autoclaved, tissues with equivalent amounts of the digestive juices and one series with Ringer's solution. All controls showed profuse bacterial growth while the others remained sterile. The toxicity of equivalent portions of the digestion products in each case was determined by intraperitoneal injection in mice. The results show that the products of the aseptic gastric and pancreatic digestion of such organs as pancreas, liver, and muscle are relatively non-toxic as compared with similar products complicated by the growth and activity of the bacteria commonly found in these tissues. These *in vitro* experiments confirm the previous observations with respect to the *in vivo* autolysis of liver and pancreas that the toxemia in each case is dependent more upon the products of bacterial activity than upon the substances of simple, non-bacterial digestion or autolysis.

5805

Effect of Celiac Ganglionectomy on Sugar Tolerance of Dogs.

GEZA DE TAKATS AND F. P. CUTHBERT. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Since the literature indicates that the output of insulin is in part regulated by the vagus and sympathetic nerves,¹ that a sympathectomized cat is hypersensitive to insulin,² and that ligation of portions of the pancreas increases the sugar tolerance of dogs³ (de Takats) it was considered important to determine the effect of removal of the celiac ganglia on the sugar tolerance.

The intravenous sugar tolerance of 3 dogs has been determined by the method of Woodyatt, Sansum and Wilder.⁴ These dogs were trained to lie quietly. Blood sugar determinations were made before, during and at intervals up to 4 hours after the injection of sugar. Urine was collected under toluol. After determining the intravenous sugar tolerance, the celiac ganglion was removed and

¹ Britton, *Am. J. Physiol.*, 1925, **74**, 291. LaBarre, *et al.*, *Compt. rend. Soc. Biol. Belge*, 1927, **96**, 193, 421, 708, 710; 1927, **97**, 917, 1048, 1406. Clark, *J. Physiol.*, 1925, **59**, 466; 1926, **61**, 576. Macleod, *Lancet*, 1930, **219**, 512.

² Dworkin, *Am. J. Physiol.*, 1931, **98**, 467.

³ de Takats, *et al.*, *Arch. Surg.*, 1929, **19**, 771; 1930, **20**, 866.

⁴ Woodyatt, Sansum and Wilder, *J. Am. Med. Assn.*, 1915, **65**, 2067.

the tolerance was determined at intervals during a period of from 5 to 10 months.

The intravenous blood sugar tolerance of the 3 dogs before operation was 1.9, 1.8, and 1.8 gm. of glucose per kilo per hour, respectively. The sugar tolerance now 5 to 10 months after removal of the celiac ganglia is 2.8, 3.4, and 3.8 gm., respectively. The sugar tolerance was increased immediately after the operation. The first determination was made at one week. In one dog the tolerance had increased from 1.8 gm. to 3.8 gm. in one week; in a second, the maximum tolerance of 2.8 gm. was not reached until one month; in the third, the tolerance was not determined until 5 months after the operation, when the tolerance was 2.8 gm.

It is to be noted that the normal tolerance values for the dog found by us are higher than those of Woodyatt, Sansum and Wilder. This may be due to the fact that we used a 5% solution of glucose, instead of a 10 to 50% solution, and in the period of one hour injected a volume sufficient to give the desired dosage per kilo per hour. We used Pfanstiehl glucose for intravenous use, and our dogs were not excited during the injection.

5806

The Titration Curve of Gastric Mucin.

K. K. JONES* AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Fogelson¹ has prepared a "gastric mucin" from the gastric mucosa of hogs which has a relatively high combining power for free hydrochloric acid and which has proved to be effective in relieving the symptoms of peptic ulcer when administered in adequate doses. Kim and Ivy² have found that the administration of "gastric mucin" prevents the development of duodenal ulcers in biliary fistula dogs. Since Fogelson did not claim that his "gastric mucin" was a pure product, since with his method of preparation, it is very likely that peptone and other products of incomplete protein digestion are present which may account for some of the acid-combining

* Josiah Macy, Jr. Foundation Fellow.

¹ Fogelson, S. J., *J. Am. Med. Assn.*, 1931, **96**, 673.

² Kim, M. S., and Ivy, A. C., *J. Am. Med. Assn.*, in press.

properties of his product, and since we were interested in the mechanism by which "gastric mucin" relieved the symptoms of ulcer and apparently promoted healing, we decided to wash Fogelson's mucin more thoroughly with 60% ethyl alcohol and compare the titration curves of the washed and unwashed "mucins".

A weighed quantity (50 gm.) of Fogelson's mucin was extracted 4 times with 500 cc. of 60% ethyl alcohol, in which peptone is soluble and mucin is insoluble. It was found that about 60% of Fogelson's mucin is soluble in 60% ethyl alcohol and 40% is insoluble.

Figure I shows the titration curves of 1 gm. each of Fogelson's

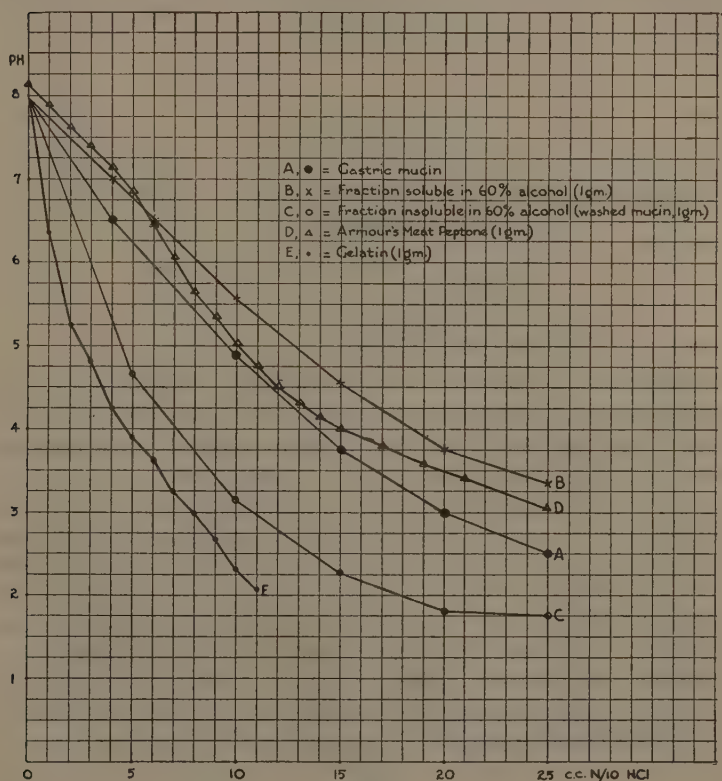


FIG. 1.

gastric mucin (A), the peptone or 60% alcohol soluble fraction (B), the mucin or 60% alcohol insoluble fraction (C), Armour's meat peptone (D), and gelatin (E), in solution in 50 cc. of distilled water. Sufficient sodium hydroxide was added to each solution to give it

a pH of 8.0 before the titration with N/10 HCl was started. This was done because it was found that the pH of different batches of Fogelson's mucin obtained from the manufacturing firm (Armour and Co.) varied from pH 7.5 to 8.0. The manufacturers are requested by Dr. Fogelson not to supply a gastric mucin powder which in solution has a more alkaline pH than 8.0. It is evident from the titration curves that a pH of 4 approximately one-half of the combining power of Fogelson's mucin is due to the presence of the peptone or 60% alcohol soluble fraction.

"Washed gastric mucin" and "gastric peptone" have been prepared in powder form (Wilson and Co.) for the purpose of testing their clinical effectiveness in peptic ulcer.

5807

Effect of Hypertonic Sodium Chloride Intravenously on Intestinal Peristalsis.

P. E. REID. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Several investigators^{1, 2, 3, 4} have found that the injection of hypertonic sodium chloride, intravenously, increases intestinal tone and motility, and Ross⁴ and Orr, Johnstone and Haden¹ have reported favorably on its clinical use in adynamic ileus. Experimentally, these investigators, by the use of balloons or direct observations, have demonstrated unequivocally that following the injection of a hypertonic salt solution the tone and motility of the small intestine is decidedly augmented. But balloon tracings and direct observation of the intestine do not decisively show that the propulsive type of motility of the intestine is augmented. That the propulsive type of motility of the intestine is increased by a certain drug or procedure can only be shown by making observations on the time of passage of a bolus through a measured length of intestine. Accordingly, we devised the following experiment to determine the effect of hyper-

¹ Orr, T. G., Johnstone, P. N., and Haden, R. L., *Surg. Gynec., and Obstet.*, 1931, **52**, 941.

² Hughson, W., and Scarff, J. E., *Johns Hopkins Hosp. Bull.*, 1924, **35**, 197.

³ Dreyer, N. B., and Tsung, Thelma, *J. Pharmacol. and Exp. Therap.*, 1929, **36**, 629.

⁴ Ross, J. W., *Canad. Med. Assn. J.*, 1926, **16**, 241.

tonic salt solution on the rate of passage of a bolus through a known length of small intestine.

A Thiry-Vella fistula of the jejunum of known length was made in 4 dogs. At various intervals throughout a period of 2 months a bolus of uniform size and shape was placed in the cephalic end of the fistula or loop and the time of passage through the loop was determined. Many preliminary tests of rate of passage through the fistula were thought necessary in order to rule out the possibility of spontaneous variations in the propulsive activity of the loop. On the day of injection of sodium chloride a preliminary test was made of the time of passage, then 10 cc. of a 5% sodium chloride solution were injected intravenously and its effect on the time of passage was followed.

TABLE I.
Passage of a bolus through a Thiry-Vella fistula, giving average rate at given times after operation.

Dog No.	Average Rate			Length of fistula post-operative
	1 week	1 month	2 months	
	min.	min.	min.	in.
1	4	4	4	6
2	4-8	4-8	20-60	6
3	10	10-15	30-60 or longer	8
4	8-12	10-20	30-60 or longer	8

Table I shows the effect of the "post operative time factor" on the passage of a bolus through the fistula in the 4 dogs. It is to be noted that in 3 of the 4 dogs the propulsive motility decreased as time elapsed. This suggests that the "disuse" of the loop of intestine tends to decrease its motility of the propulsive type which is opposite to the effect of "disuse" on the general postural tone of the loop, since Dr. Ivy informs me that the postural tone of an isolated stomach or loop of the intestine increases in postural tone as time elapses. This further indicates that we must distinguish between, or it might be best to divide intestinal motility into 2 general types, namely, propulsive motility and non-propulsive motility. In this connection, we might add that pilocarpine (2-3 mg.) and morphine (15 mg.) in the doses we used increased the postural tone and rhythmic activity of the loop, but did not increase the rate of passage of the bolus.

Table II shows that intravenous injection of 10 cc. of 5% NaCl solution increased the propulsive motility of the jejunal loop in 3 of the 4 dogs, the exception being the dog in which the "post operative time factor" did not lead to a decrease in propulsive motility.

TABLE II.

Effect of intravenous hypertonic 10 cc. 5% NaCl on propulsive motility of a Thiry-Vella fistula of the jejunum.

Dog No.	Oct. 21, 1931			Oct. 25, 1931			Oct. 28, 1931	
	Normal Time	After NaCl	Latent period or NaCl effect	Normal Time	After NaCl	Latent period or NaCl effect	Norm. Time	After NaCl
1	min. 4	min. 4	—	min. 4	min. 4	—	min. 4	min. 4
2	30 to go	4	6	10	5	6	7	4
3	$\frac{1}{2}$ way							
	"	4-6	7	20	10	6	12	10
4	"	8	6-8	15	10	6	13	11

It might be said that, in this dog, propulsive motility was maximum and hence could not be influenced by the salt solution. That this explanation is probable, is supported by the fact that the effect of the injection of salt solution lasts at least one week, and that an injection of salt solution during this period failed to cause a marked increase in propulsive motility.

In regard to the action of the hypertonic salt solution on the motility of the colon and stomach, we can only at this time state that it does not cause defecation, and that it increases the tone and motility of the stomach as recorded by a balloon. In order to answer this question specifically, study is under way to determine the effect of hypertonic sodium chloride solution on the evacuation time of the colon and stomach.

Conclusion. The intravenous injection of 10 cc. of 5% NaCl solution increases the propulsive type of motility of a jejunal loop when the loop is manifesting subnormal propulsive motility.

5808

Observations on the Metabolism of the Tapeworm, *Moniezia Expansa*.

HOWARD L. ALT AND OTTO A. TISCHER. (Introduced by C. J. Farmer.)

From the Departments of Chemistry and Medicine, Northwestern University Medical School.

The association of the tapeworm with an Addisonian type of anemia in the human has been repeatedly described. The tapeworm may destroy a specific nitrogenous substance necessary for blood regeneration.¹ Observations on the respiration and fermentation of

¹ West, Randolph and Howe, Marion, *J. Am. Med. Assn.*, 1931, 97, 685.

various portions of the sheep tapeworm are reported here preliminary to further studies on the protein metabolism of tapeworms pathogenic to man.

Observations on the respiration, respiratory quotient, and anaerobic and aerobic acid formation of the sheep tapeworm were made, using the technique of Warburg.² Small quantities (2 to 40 mg. dry weight)* of the head portion, and of the mature and gravid proglottides were studied in phosphate-Ringer or bicarbonate-Ringer solution with 0.2% glucose at a pH of 7.4 and at 37.5°C.

The oxygen consumption decreased for the first 2 hours and then assumed a constant value which continued as long as readings were made (4 to 5 hours). The initial values were about twice those of the values during the steady state. The average values for the Q_{O_2} in 8 experiments during the steady state were 1.1 for the head portion, 0.9 for the mature proglottides, and 0.6 for the gravid proglottides. Oxygen consumption was the same under the following conditions: in 100% oxygen, in room air, in solutions with and without glucose, in an intact portion of the worm as compared with an equal adjacent portion when divided into several groups of proglottides.

Four determinations of the respiratory quotient during the first hour gave values of 1.21 and 0.90 for the mature proglottides and 1.00 and 1.16 for the gravid proglottides. The technique was exactly as outlined by Richardson³ except that 10% citric acid was used to liberate the carbon dioxide. Accurate values could not be obtained because of the relatively small oxygen consumption as compared with the large amount of bound carbon dioxide in the worm.

The anaerobic acid formation of the mature and gravid proglottides was constant for the first 1½ to 2 hours and then decreased slightly for the following 2 hours. The average $Q_{CO_2}^{N_2}$ in 9 experiments was 5.2 for the mature and 3.8 for the gravid proglottides. The initial $Q_{CO_2}^{N_2}$ of 9.4 for the head portion fell and reached a constant of 5.9 at the end of the second hour. This is explained by the fact that the head portion (about 15 cm. long) which was very motile at first, tied itself into knots and became relatively immotile. When glucose was omitted from the media, there was a decrease in

² Warburg, Otto, *Über den Stoffwechsel der Tumoren*, 1926, J. Springer, Berlin.

* The dry weight is approximately 10% of the moist weight.

³ Richardson, H. B., *Physiol. Rev.*, 1929, 9, 61.

acid formation after the second hour in the case of the mature and gravid proglottides. In the case of the head portion, the decrease in acid formation was noticed after the first half hour. The acid formation was the same in an intact portion of worm as in an equal adjacent portion when divided into several groups of proglottides.

The acid formation is practically the same either in presence of a 95% oxygen mixture or in absence of oxygen. The table shows the result of a typical experiment, performed in rectangular vessels by the improved method of Warburg.²

TABLE I. *Mature Proglottides.*

min.	QO ₂	Q $\frac{O_2}{CO_2}$	Q $\frac{N_2}{CO_2}$
30	—2.8	+4.2	+4.0
30	—2.8	+3.9	+3.9
30	—2.0	+3.6	+3.9

QO₂ = cmm. of O₂ consumed per mg. of dried worm per hour.

Q $\frac{O_2}{CO_2}$ = cmm. of extra CO₂ liberated per mg. per hour in oxygen.

Q $\frac{N_2}{CO_2}$ = cmm. of CO₂ liberated per mg. per hour in nitrogen.

It has been found that lactic acid and fatty acids, in almost equal quantities make up the majority of the acids produced in the metabolism of the sheep tapeworm.^{4,5} Small amounts of succinic acid⁵ and oxalic acid⁶ have also been reported.

5809

A Correlation Study of Vasomotor and Muscle Tonus Response to Sympathetic Ganglionectomy.

J. DEWEY BISGARD. (Introduced by L. R. Dragstedt.)

From the Department of Surgery, Division of Orthopedics, University of Chicago.

The theory of dual motor innervation of striated muscle suggested by the histological studies of Boeck¹ and supported by the physiological observations of DeBoer² and Langelaan³ has led to considerable

⁴ Tischer, O. A., unpublished work.

⁵ Brandt, T., *Verhandl. d. deutsch. zoolog. Gesellschaft*, 1929.

⁶ Loeper, M., and Tonnet, J., *Comp. rend. Soc. de biol.*, 1931, **106**, 716.

¹ Boeke, J., *Brain*, 1921, **44**, 1.

² Boer, S. de, *Folia Neuro-biol.*, 1913, **7**, 378.

³ Langelaan, J. W., *Brain*, 1915, **38**, 235.

experimental investigation and controversy. Assuming this theory and the functional hypothesis that the sympathetic nervous system was responsible for the maintenance of plastic tone and that the medullated or somatic fibers served only to initiate contractile tone, Hunter⁴ and Royle⁵ proceeded experimentally, and later clinically, to give these studies clinical application. They interrupted the sympathetic innervation of one extremity of animals upon which an upper motor neurone (decerebrated spasticity) type of paralysis had been or was subsequently produced, and reported hypotonicity of the muscles of the sympathetomized side. However, no one has been able to duplicate their results experimentally or clinically (Von Lochem excepted).

Recently, Wilkinson⁶ has demonstrated quite conclusively that striated muscle tissue receives no sympathetic innervation. Cobb,⁷ Tower,⁸ Kanavel and Davis,⁹ Kuno,¹⁰ and others were unable to show any constant difference in muscle tone as a result of sympathetic deinnervation. If the animals were observed from time to time the normal (control) extremity would show diminished tone as frequently as the operated side.

In an attempt to correlate the definitely recognized interruption of vasomotor control to any alteration in muscle tone (if obtainable) these classical experiments were repeated and frequent skin temperature determinations made. These readings have been tabulated for 2 animals. All the methods commonly used to produce hypertonicity were used. Strychnine was used for 16 observations on 10 animals; parathyroid tetany was induced in 2 animals; ether rigidity 34 times in 20 animals; decerebrated rigidity in 4 animals and spinal cord section in 2 animals.

From 14 animals (10 dogs and 4 goats) the lumbar ganglia 2, 3, 4, and 5 and their corresponding trunks were removed on the left side. In 6 animals (4 dogs and 2 goats) the left stellate ganglia were removed. This latter group all developed typical Horner's syndromes. In most instances the sympathetic chains were exposed on both sides so as to preclude an error in interpretation of results caused by the trauma of the operative approach. Muscle tone

⁴ Hunter, J. I., *Surg. Gynec. and Obstet.*, 1924, **39**, 721.

⁵ Royle, N. D., *Surg. Gynec. and Obstet.*, 1924, **39**, 701.

⁶ Wilkinson, *Med. J. Australia*, 1929.

⁷ Cobb, S., *Physiol. Rev.*, 1925, **5**, 518.

⁸ Tower, S. S., *Am. J. Physiol.*, 1926, **78**, 462.

⁹ Kanavel, A. B., Pollock, L. J., and Davis, L. E., *Arch. Neurol. and Psych.*, 1925, **13**, 197.

¹⁰ Kuno, Y., *J. Physiol.*, 1914, **49**, 147.

was measured by the maintenance of position against gravity, by the resistance offered to passive extension and flexion and a comparison of reflexes. With the exception of one dog there appeared to be no constant difference in muscle tone on the 2 sides; comparing the normal and sympathectomized extremities. This one animal, showing evidence of marked vasomotor reactions in the unoperated extremity was observed in ether rigidity 5 times; in strychnine rigidity twice and decerebrated rigidity once. During all these observations diminished muscle tone was manifested quite consistently in the sympathectomized extremity. While in the decerebrated state, ligatures were placed around the axillary vessels on both sides and within 5 minutes both extremities relaxed and no difference in tone could be demonstrated.

These observations and the observations made by Tower of constant hypotonicity in 2 of her series of 15 decerebrated animals would seem to indicate that any alteration of muscle tone (when-ever demonstrable) is dependent upon the alteration of the blood flow through the muscle tissue resulting from the removal of vasomotor control.

Minnesota Section.

University of Minnesota, October 21, 1931.

5810

Gall Bladder Evacuation by Egg Yolk in Stomach.

JAMES E. MORGAN, LATHAN A. CRANDALL, JR., AND ANDREW C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Drewyer and Ivy¹ have reported that cholecystokinin can be extracted in relatively small quantities from the gastric mucosa. To determine whether this hormone could be liberated in effective amounts from the stomach alone, 5 dogs were prepared with pouches of the entire stomach and direct anastomoses between the esophagus and duodenum. Following visualization of the gall bladders of these animals by tetraiodophenolphthalein the stomachs were lavaged for from 2 to 3 hours with the yolks of 4 eggs. In 2 of the dogs the gall bladders were found to be empty after 2 hours of such lavage; this was repeated and confirmed in one of these. In the other 3 animals no emptying occurred, although the lavage was continued for 3 hours; a second experiment in one of these animals again showed no emptying. In the 3 dogs whose gall bladders did not empty from egg yolk in the isolated stomach, the administration of the yolk by mouth produced evacuation, although in one case the gall bladder still contained a small amount of dye after 6 hours.

The positive results in 2 of the dogs indicate that the presence of fat in the stomach alone may in some cases liberate into the blood stream an amount of cholecystokinin adequate to induce gall bladder contraction. The fact that the splanchnic nerve supply to these stomach pouches was intact (vagi severed) makes it possible that the emptying may have been due to a reflex set up by contact of the egg yolk with the gastric mucosa, but this possibility is considered rather remote.

¹ Drewyer, G. E., and Ivy, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1929, **27**, 186.

Gall Bladder Visualization in Experimental Achylia.

JAMES E. MORGAN, LATHAN A. CRANDALL, JR., AND ANDREW C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Hines¹ has reported 11 cases of *Achylia gastrica* in which the Graham test was done; these patients received the tetraiodophenolphthalein intravenously. In most of these cases the gall bladder failed to visualize and in the others the shadow was reported as abnormal. Patients with absence of shadow showed visualization in some instances when HCl was given by mouth. More recently Davis and Talley² have reported 20 cases of anacidity, in the majority of which they obtained good visualization although 9 of these cases presented clinical evidence of gall bladder disease. They gave the dye by mouth.

To determine whether the absence of hydrochloric acid from the gastrointestinal tract was capable of interfering with gall bladder visualization, 5 dogs with pouches of the entire stomach (esophago-duodenal anastomosis) in which all of the gastric secretion drained to the outside were subjected to the Graham test. Tetraiodophenolphthalein, 0.18 gm. per kilo, was given intravenously and 10 to 12 hours later roentgenograms were made of the gall bladder area. A total of 7 such experiments were performed on the 5 animals. In all cases the gall bladder showed normal concentration of the dye. In 4 instances egg yolk was given by mouth and evacuation of the gall bladder followed by x-ray. The gall bladder emptied promptly in 3 cases; in the fourth contraction occurred but complete evacuation was delayed more than 6 hours.

Although acid in the duodenum is undoubtedly a factor in evacuation of the viscus, the above results indicate that gall bladder function, in the dog at least, is not impaired by the presence of complete achylia.

¹ Hines, L. E., *J. Am. Med. Assn.*, 1928, **90**, 2099.

² Davis, D., and Talley, D. D., *J. Am. Med. Assn.*, 1929, **92**, 110.

5812

Effect of Sympathetic Ganglionectomy Upon Bone Growth.

J. DEWEY BISGARD. (Introduced by L. R. Dragstedt.)

From the Department of Surgery, Division of Orthopedics, University of Chicago.

The possibility of increasing the rate of bone growth in an extremity by the interruption of its sympathetic innervation has received clinical trial by Harris^{1, 2} and others in recent years in cases with residual paralysis from anterior poliomyelitis in which there has been retardation of growth and a discrepancy in length in the 2 extremities. It has been assumed that the removal of vasomotor control would sufficiently increase the blood supply to the epiphyseal growth centers to bring about accelerated activity.

All experimental investigation conducted upon normal animals has produced negative results. Cannon³ and his coworkers have removed the entire sympathetic chain on one side of the body of kittens and infant rats and have observed no increase in growth on the sympathectomized side of the component structures of the extremities, the bilateral symmetrical soft tissues and viscera with the exception of the reproductive organs. René Simon⁴ has made the same observations on rabbits.

These investigators apparently read the results of their experiments only at the conclusion of a growth period, that is at post mortem examination. To preclude the possibility of a temporary acceleration of bone growth at some stage in development, the left lumbar sympathetic chains (ganglia 2, 3, 4, and 5 and intervening trunks) were removed from 4 kid goats weighing approximately 6 kg. each and frequent periodic roentgenograms were made of both lower extremities.

At present, these animals have been observed for 6 months and during this period their weight has quadrupled and the tibiae on both sides have increased in length from 6 to 6.5 cm. Bimonthly examinations, however, have demonstrated that the tibiae on the sympathectomized sides have at no time evidenced an increased rate of growth in comparison with the controls of the normally innervated extremities.

To these experiments the objection can be raised that they were

¹ Harris, R. I., *J. Bone and Joint Surg.*, 1930, **12**, 859.

² Bacq, Z. M., *Am. J. Physiol.*, 1930, **95**, 601.

³ Cannon, W. B., Newton, H. F., Bright, E. M., *Am. J. Physiol.*, 1929, **89**, 84.

⁴ Simon, R., *Compt. Rend. Soc. Biol.*, 1930, **108**, 709.

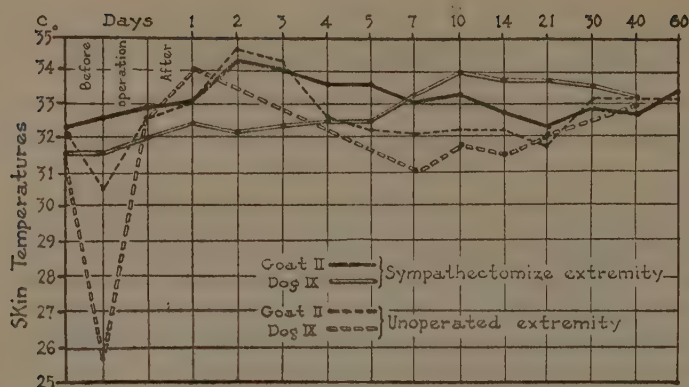


FIG. 1.

conducted upon normal animals and to overcome this objection a precisely parallel experiment to the human cases of residual paralysis in anterior poliomyelitis was sought, and from Doctor Hudson of the bacteriological department of the University of Chicago a half grown monkey with open epiphyses and complete symmetrical residual paralysis of both lower extremities from anterior poliomyelitis was obtained. The same procedures have been carried out upon this animal, but too short a time has elapsed to draw any definite conclusions. At present (2 months after the operation) the growth (0.2 cm. in both tibiae) has been too small to permit accurate comparative measurements.

5813

Fiber Connections of Clarke's Column (Nucleus Dorsalis)

I. J. PASS. (Introduced by A. T. Rasmussen.)

From the Department of Anatomy, University of Minnesota.

The anatomical and functional relationships of Clarke's column (*Nucleus dorsalis*) have been long disputed. Several men, upon the basis of comparative studies, have argued that it is visceral sensory. In fishes, this cell column connects with a well demonstrated visceral sensory nucleus in the cerebellum. Furthermore, Clarke's column is found in the thoracic and upper lumbar levels, just where visceral afferent fibers are relayed into the cord via the white rami.

Most modern authors, however, assume that Clarke's column is somatic afferent because it contributes fibers to the spino-cerebellar tracts which terminate in the cerebellar cortex.

The real nature of Clarke's column must be determined by its peripheral connections as well as by the termination of the fibers arising from it. In connection with the first point, we have found that section of the dorsal roots of the large nerves of the hindlimb of the cat (which contain practically no visceral afferent fibers), shows by the Marchi method, after 10 days to 2 weeks of degeneration, many fine degenerated fibers swinging into the lower end of Clarke's column.

In regard to the second point, we have succeeded in obtaining in several cats, small well-circumscribed lesions which throw light upon the course of the fibers leaving Clarke's column. In one cat we were so fortunate as to destroy completely one nucleus without involving any adjacent gray matter. Marchi preparations of this animal's cord showed numerous degenerated fibers swinging out toward each lateral column. Sections above the lesion showed these fibers becoming concentrated in the spino-cerebellar tracts which terminate largely in the vermis. A second cat in which parts of both nuclei alone were destroyed, gave similar results. These facts considered together, support the proprioceptive nature of Clarke's column, unless one postulate the existence of a cerebellar visceral sensory nucleus in the cat.

Our work further clarifies the problem of the course of the fibers arising from Clarke's column. A unilateral lesion, destroying one nucleus and passing ventrally to the central canal so as to interrupt all fibers crossing from the opposite nucleus, gave equal numbers of degenerated fibers in Flechsig's tract of each side, indicating, apparently, that all the fibers arising from this cell column in the cat cross to the opposite side. This conclusion was further verified by a lesion which passed between the 2 nuclei without destroying any significant part of either one, but completely interrupting the dorsal gray commissure. This lesion resulted in equal numbers of degenerated fibers on both sides of the cord. The number of fibers was equal to that obtained from a complete destruction of the nucleus of one side, plus the dorsal gray commissure over the same longitudinal extent and at the same level of the cord.

5814

Effects of Castration Leaving the Epididymis Intact in the Rat.

J. J. LAWLESS. (Introduced by A. T. Rasmussen.)

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Recently Gallagher¹ found that he could stimulate comb growth in capons by the injection of an extract of epididymis just as he could by the injection of a similar testicular extract. Similar extracts of other organs were ineffectual. This would indicate that the epididymis possesses some endocrine activity similar to that of the testis.

I have removed the testes in a series of 11 three-weeks old albino rats, the epididymides being left intact. Ten litter-mates of these rats served as controls. At the age of 80 days, the animals were chloroformed and autopsied. The body weight, nose-anus length, and the weights of the various organs were determined.

These data for the test animals were compared with the corresponding data for the control animals. It was found that the experimental procedure had produced the following effects:

1. Body length was slightly less in the test animals (-0.878 ± 0.328 cm.). This difference may be too little to be considered as significant.
2. Body weight was not affected. (-0.743 ± 8.837 gm.)
3. The weight of the hypophysis was markedly increased. ($+4.898 \pm 0.266$ mg.)
4. The weight of the adrenals was increased. ($+5.674 \pm 1.348$ mg.)
5. The weight of the thymus was slightly, perhaps not significantly, increased. ($+0.126 \pm 0.044$ gm.)
6. The weight of the spleen was unaffected. (-0.021 ± 0.038 gm.)
7. The accessory reproductive organs were distinctly atrophied.
8. The weight of the kidneys was decreased. (-0.377 ± 0.104 gm.)
9. The liver was reduced in weight. (-1.920 ± 0.553 gm.) (No previous report concerning the effect of castration on the weight of liver was found.)
10. The weight of the brain was not changed. (-0.00068 ± 0.090 gm.)

My results are similar to those reported by previous workers who castrated their animals in the ordinary way; provided, that the comparison is made with rats castrated at about the age of 21 days, and in which the body and organ weights were determined at approximately 80 days.

The epididymis, therefore, apparently does not produce an internal secretion capable of maintaining normal size in these organs when the testes are absent.

¹ Gallagher, *Am. J. Physiol.*, 1928, **87**, 447.

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Effects of Posterior Pituitary Extracts on the Oxygen Consumption of Excised Tissue.

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It has been noted that pituitrin and pitressin,¹ when injected subcutaneously cause a diminished oxygen consumption of rats. Injections of pitocin, on the other hand, were followed by a slight increase of the intake of oxygen. The action of the posterior pituitary extracts may be either of central or peripheral origin, or both. Therefore it becomes of interest to determine the effects of these extracts on excised tissues.

Rats weighing approximately 250 gm. were used and diaphragm, liver, and testis were studied according to the technique of Warburg.² In each experiment, 8 pieces of tissue of the same organ were used; 4 with one of the posterior pituitary extracts and 4 without. Any determination which diverged widely from the others was discarded. Thus each value given in the table represents the best check of 4 determinations. The extracts were kindly furnished by Dr. Oliver Kamm and contained 10 pressor units (pitressin), 10 oxytocic units (pitocin) or 10 units of each of these fractions (pituitrin), per cc. The extracts were freed of chloratone before use, and the total volume of fluid in the respiration chamber was 3 cc.

TABLE I.
Oxygen consumption of excised tissues in cu. mm. per mg. and minute.

	Pituitrin 1/4 cc.		Pitressin 1/10 cc.		Pitocin 1/4 cc.	
	with	without	with	without	with	without
Testicle	.008	.026	.011	.042	.003	.003
Diaphragm	.007	.024	.012	.014	.018	.027
Liver	.020	.045	.013	.065	.009	.012
	Pituitrin 1/10 cc.		Pitressin 1/20 cc.		Pitocin 1/20 cc.	
	with	without	with	without	with	without
Testicle	.004	.019	.001	.017	.009	.011
Diaphragm	—	—	.019	.048	.003	.017
Liver	—	—	.054	.064	.008	.015

Conclusions. The action of pituitrin and pitressin is to depress the oxygen consumption of excised tissue while that of pitocin is not consistent. Further work is being done with these extracts.

¹ Himwich, H. E., and Haynes, F. W., *Am. J. Physiol.*, 1931, **96**, 640.

² Warburg, O., *Biochem. Z.*, 1923, **142**, 317.

Effects of Acetylcholine Injections on Blood Fat and Glucose.

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Recent evidence shows that parasympathetic effects are mediated by acetyl choline.¹ Stimulation of the vagus (a parasympathetic nerve) results in a decrease of blood glucose,² presumably because of increased insulin production. Insulin itself produces a decrease in blood fat and glucose.³ It is of interest, therefore, to determine the effect of injections of acetyl choline upon blood fat and glucose.

Four injections of 1 to 2 mg. acetyl choline per kilo of body weight, a half hour apart were made into the superior pancreato-duodenal artery of dogs under amytal anesthesia. Blood glucose determinations by the Hagedorn-Jensen method, and blood fat determinations by the Stewart and White method as modified by Himwich, Friedman and Spiers⁴ were made upon blood samples drawn from the femoral artery.

TABLE I.

Effect of injections of acetyl choline into pancreato-duodenal artery upon blood fat and glucose—mg.%.

	Per injection	2 hr.	4 hr.	6 hr.
(1) glucose	102	65	60	70
fat	674	636	512	571
(2) glucose	104	104	89	87
fat	673	647	602	648
(3) glucose	95	87	77	78
fat	1022	727	682	758

Conclusion. Injections of acetyl choline into the pancreato-duodenal artery causes a decrease in blood fat and glucose.

¹ Dale, H. H., and Gaddum, J. H., *J. Physiol.*, 1930, **70**, 109.

² Britton, S. W., *Am. J. Physiol.*, 1925, **74**, 291.

³ Himwich, H. W., and Spiers, M. A., *Am. J. Physiol.*, 1931, **97**, 648.

⁴ Himwich, H. E., Friedman, H., and Spiers, M. A., *Am. J. Physiol.*, 1931, in press.

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The Degree of Saturation of Blood Fats Mobilized During Diabetes.*

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It is known that a lipemia may occur in diabetes. Depancreatized dogs develop a lipemia which is more marked and appears earlier than that of phlorhizinized dogs.¹ However, the character of the fatty acids mobilized has not been determined. Hence in the present study the iodine numbers have been ascertained on 12 depancreatized and 7 phlorhizinized dogs. Iodine number and total fat content of each sample of blood were estimated, the former according to the modification of Gibson and Howard² and the latter according to that of Himwich, Friedman and Spiers.³ An increase in the blood fat was always accompanied by a decrease in the iodine number. Typical experiments are presented in Table I.

TABLE I.

Blood fat and iodine numbers before and after pancreatectomy or injections of phlorhizin—mg. %.

Time	Phlorhizin		Pancreatectomy	
	total fat	iodine numbers	total fat	iodine numbers
Before	556	118	507	83
1 day	735	105	993	52
2 days	717	94	1494	46
3 "	1031	57	2508	33
4 "	1022	65		

Since the iodine number depends on the fraction $\frac{\text{iodine absorbed}}{\text{total fatty acids}}$ the diminution of the iodine number indicates that the amount of unsaturated fatty acids does not vary as much as the saturated, *i. e.*, saturated rather than unsaturated fatty acids are mobilized.

* The expenses of this investigation have been defrayed, in part, by a Grant-in-Aid of the American Medical Association.

¹ Himwich, H. E., Chambers, W. H., Hunter, A. L., and Spiers, M. A. To appear in the *Am. J. Physiol.*, 1931.

² Gibson, R. B., and Howard, C. P., *Arch. Int. Med.*, 1923, **32**, 1.

³ Himwich, H. E., Friedman, H., and Spiers, M. A. To appear in the *Biochem. J.*, 1931.

Effect of Nocuous Stimulation on Blood Fat.

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Cannon¹ showed that the liberation of adrenalin into the blood stream is increased by nocuous stimulation. Since injections of adrenalin² and emotional excitement, *i. e.*, fear and rage³ produce lipemia, it was logical to determine whether nocuous stimulation applied to the pads of the feet of cats would have the same effect.

Blood was drawn from the jugular vein of cats before and after a period of electrical stimulation of short duration (less than 2 minutes), and the samples were analyzed for fat and glucose. The results presented in Table I indicate that nocuous stimuli produce a reflex lipemia.

TABLE I.
Effect of nocuous stimulation on blood fat—mg. %.

Experiment	Fat		Glucose	
	before	after stimulation	before	after stimulation
1	678	862	114	154
2	644	662	148	161
		735 ($\frac{1}{2}$ hr.)		204 ($\frac{1}{2}$ hr.)
3	574	681	144	184
4	825	1040	192	280

¹ Cannon, W. B., "Bodily changes in pain, hunger, fear and rage," New York, D. Appleton and Company, 1929.

² Himwich, H. E., and Spiers, M. A., *Am. J. Physiol.*, 1931, **97**, 648.

³ Himwich, H. E., and Fulton, J. F., *Am. J. Physiol.*, 1931, **97**, 533.